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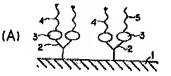
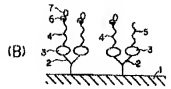
(54) METHOD FOR MEASURING RATIO OF SPECIFIC SUGAR CHAIN OF GLYCOPROTEIN

(57)Abstract:
PURPOSE: To provide a method for measuring the ratio of quantity of the specific sugar chain of the specific glycoprotein, which can easily and accurately measure the ratio of quantity of the specific sugar chain to the whole of the sugar chain of the specific glycoprotein in the sample without separately measuring the total of the sugar chain of the specific glycoprotein.
CONSTITUTION: A solid phase 1, to which a constant quantity of the antibody 2 having the specific compatibility with a protein part of a specific glycoprotein 3, and a liquid phase of the sample, which includes the specific glycoprotein 3, are made to contact with each other, and the specific glycoprotein 3 is supplied to the antibody 3 till the antibody is saturated with the specific glycoprotein 3. Lectin 6 labeled by the labeled material 7 having the compatibility with the specific sugar chain 4 of the specific glycoprotein 3 is connected so as to measure the quantity of the labeled material, and the quantity of the labeled material is compared with the detected quantity value data, which shows the relation of the quantity of the labeled material obtained by using the standard sample, of which ratio of the specific sugar chain is already known, with the ratio of the specific sugar chain, so as to obtain the ratio of the specific sugar chain.





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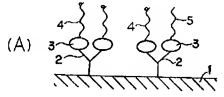
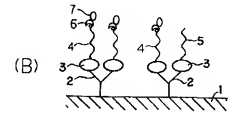
(54)【発明の名称】　糖タンパク質の特定糖鎖割合の測定方法

(57)【要約】

【目的】　特定糖タンパク質の糖鎖総量を別途測定する必要がなく、試料中の特定糖タンパク質の全糖鎖量に対する特定糖鎖量の割合を簡便かつ迅速に測定することができる特定糖タンパク質の特定糖鎖量の割合の測定方法を提供する。

【構成】　特定糖タンパク質３のタンパク質部分に特異的親和性を有する抗体２の一定量が固定された固相１と、前記特定糖タンパク質３を含有する試料の液相とを接触させて、抗体２に該特定糖タンパク質３を飽和状態まで捕捉させ、次いで該糖タンパク質３の特定糖鎖４に対して親和性を有する標識物７で標識化されたレクチン６を結合させ、その標識物量を測定し、該標識物量を、特定糖鎖の割合が既知である標準試料を用いて求めておいた該標識物量と特定糖鎖の割合との関係を示す検量値データと比較することにより特定糖鎖割合を求める。





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【特許請求の範囲】

【請求項１】　（ａ）特定糖タンパク質のタンパク質部分に特異的親和性を有する抗体および／または該抗体の活性フラグメントの一定量を固定した固相と、前記特定糖タンパク質を含有する試料の液相とを接触させることにより、該固相上の該抗体および／または該抗体の活性フラグメントに該特定糖タンパク質を飽和状態まで捕捉させた後、固相と液相を分離し、次いで分離した固相と該糖タンパク質の特定糖鎖に対して親和性を有する標識化されたレクチンを含有する液相を接触させ、該固相上に捕捉されている特定糖タンパク質の特定糖鎖に標識化されたレクチンを結合させ、次いで固相と液相を分離し、分離した固相にレクチンを介して結合している標識物量を測定し、該標識物量を

（ｂ）一定量の該特定タンパク質が含有する全糖鎖量に対する特定糖鎖の割合が既知である標準試料を用いて前記（ａ）と同様の方法により同様の標識物量の測定を行い、その結果に基いてあらかじめ作成された該標識物量と該特定タンパク質が含有する全糖鎖量に対する特定糖鎖の割合との関係を示す検量値データと比較することにより前記試料に含有されている該特定糖タンパク質の全糖鎖量に対する特定糖鎖割合を決定することを特徴とする特定糖タンパク質の全糖鎖量に対する特定糖鎖量の割合の測定方法。

【請求項２】　固相に固定した抗体および／または該抗体の活性フラグメントが、測定対象となる特定糖鎖を含まない抗体および／または該抗体の活性フラグメントである請求項１に記載の特定糖タンパク質の全糖鎖量に対する特定糖鎖割合の測定方法。

【請求項３】　特定糖タンパク質を含有する試料が、採取された尿、血液、血漿、血清から選ばれた１種であって、固相に固定化した抗体および／または該抗体の活性フラグメントのすべてに特定糖タンパク質が結合する濃度以上で該特定糖タンパク質を含有する試料である請求項１または２に記載の特定糖タンパク質の全糖鎖量に対する特定糖鎖割合の測定方法。

【請求項４】　標識化されたレクチンが、酵素またはビオチンのいずれかで標識されたレクチンである請求項１～３のいずれかに記載の特定糖タンパク質の全糖鎖量に対する特定糖鎖量の割合の測定方法。

【発明の詳細な説明】

【０００１】

【産業上の利用分野】本発明は、特定糖タンパク質の全糖鎖量に対する特定糖鎖量の割合の測定方法に関する。

【０００２】

【従来の技術】糖タンパク質は、アミノ酸を構成成分とするタンパク質と糖を構成成分とする糖鎖が結合した天然高分子であり、生体内に存在するタンパク質の大部分は糖タンパク質である。糖タンパク質の糖鎖は、ガラクトース、Ｎ－アセチルグルコサミン、マンノース、フコース等の糖が分枝状に連結したものであり、その糖鎖構造は生体内の生合成や代謝に際して各物質の伝達となる重要な役割を果たしている。この糖タンパク質の糖鎖構造と疾患との関係が詳細に研究され、癌や慢性関節リウマチにて健常人と異なった糖鎖構造を有する糖タンパク質の出現が報告されるようになり、臨床診断に際しても糖鎖構造分析は重要となりつつある。以下、慢性関節リウマチを例として臨床診断の現状と疾患に伴う糖鎖構造変化について詳細に説明する。

【０００３】慢性関節リウマチ（以下ＲＡと略す）は、多発性の関節炎を主とする疾患であり、膠原組織の炎症性変化を示す自己免疫疾患の一種である。ＲＡの診断は、臨床的特長を主として、これに免疫学的要因としてのリウマチ因子（以下ＲＦと略す）量が加味されて行われている（廣瀬俊一、医学と薬学　３０巻、３３ページ１９９３年）。

【０００４】ＲＦはヒト免疫グロブリンＧ（以下ＩｇＧと略す）の一部の部位を抗原として認識する抗体であることが知られており、ＲＦ測定にはヒトＩｇＧをラテックスに吸着させた試薬と患者血清とを混合し、凝集反応を調べるシンガー・プロッツ（Ｓｉｎｇｅｒ・Ｐｌｏｔｚ）法やヒト免疫グロブリンをヒツジ赤血球に吸着させた試薬と患者血清とを混合し、凝集性を見るワーラー・ローズ（Ｗａａｌｅｒ・Ｒｏｓｅ）法などが用いられている。しかしＲＦは、肝疾患、癌患者など他の疾患患者にも高頻度に存在し、健常人の３～７％にも確認されることより、ＲＡ患者におけるＲＦ存在の特異性は低い。したがって、臨床現場でＲＡ診断の指標として測定されているＲＦは、必ずしもＲＡに特異性の高い診断指標となっていない。

【０００５】最近、血清中のＩｇＧに存在する糖鎖について詳細に研究が行われ、ＩｇＧ上糖鎖末端ガラクトース残基の存在率がＲＡ患者で有意に減少している事が報告された（Ｒ.Ｂ. Parekh et al. Nature 316　452（1985））。すなわち、ＩｇＧの糖鎖末端ガラクトース残基が２分子とも欠損している割合は、健常人では全糖鎖の約２５％であるのに対し、ＲＡ患者では約５０％にまで増加していたと報告された。そして、糖鎖末端ガラクトース残基が欠損することにより、ＩｇＧに抗原性があらわれ、ＲＦを導くことが予想されている。更にこの糖鎖末端ガラクトースの欠損は、ＲＡの薬物治療が原因となって生じた２次的な現象ではなく、ＲＡ患者に普遍的に起こる特異的な現象である事が指摘されている。（信沢孝一編集「複合糖質」蛋白質核酸酵素　臨時増刊号　第１９２４～１９６２頁　平成３年８月１０日発行　共立出版（株））。よって、ＩｇＧの糖鎖末端ガラクトース欠損の検出はＲＡ診断の新しい指標となる可能性が有るとして、その測定法の開発が期待されている。

【０００６】以上のように、病態と糖タンパク質の糖鎖構造変化に密接な関係を見いだせる場合、特定糖タンパ

ク質の全糖鎖量に対する特定糖鎖量の割合の測定値は臨床判断に有用な指標となる可能性が有るとして、定量方法の開発が進められている。

【０００７】糖タンパク質の糖鎖を測定する方法としては、薄相クロマトグラフィー、ゲル電気泳動、ガスクロマトグラフィー、液体クロマトグラフィー、アフィニティークロマトグラフィーなどがあり（新生化学実験講座３糖質Ｉ、糖タンパク質、１９９０年５月２１日発行、東京化学同人）、広く糖タンパク質の糖鎖解析に利用されているが、測定感度、操作性、検体処理時間などを考慮すると、これらの方法をそのまま臨床検査法として利用することは困難である。この問題を解決する方法として、レクチンを利用する測定方法が考えられた。レクチンは、特定の糖鎖構造を認識して特異的な親和性を示すタンパク質であり、糖鎖認識能については成書（新生化学実験講座３糖質Ｉ、糖タンパク質、１９９０年５月２１日発行、東京化学同人、第９～１８頁）に詳しく説明されている。

【０００８】特開昭６１－２０８６７公報は、目的の糖タンパク質に特異的親和性を有する抗体で特定糖タンパク質を濃度依存的に捕捉し、捕捉された特定糖タンパク質の糖鎖量を標識レクチンで検出する抗体－レクチンのサンドイッチ法を報告しているが、実施例１．Ｖ．標準の尿の調製にて、標準品を緩衝液で希釈調製していることから解るように、本法は抗原濃度に依存する糖残基量を測定する方法であり、抗原の全糖鎖量に対する特定糖鎖量の割合を測定する方法ではない。

【０００９】特開平４－１３０２７４公報は、特定糖タンパク質の糖鎖と親和性のあるレクチンで目的の糖タンパク質を濃度依存的に捕捉し、さらに目的の糖タンパク質の糖鎖と親和性を有する標識レクチンで検出するレクチン－レクチンサンドイッチ法を提案しているが、レクチンの反応は抗原抗体反応と比較して特異性が低く、特定糖タンパク質の全糖鎖量に対する特定糖鎖量の割合を正確に測定することは不可能である。

【００１０】ＲＡ関連のレクチンを用いた特定糖鎖濃度測定方法に関し、特開平３－７３８５７公報は、糖鎖の末端ガラクトース残基が欠損したＩｇＧ量を測定する方法を提案している。この方法は、プロテインAあるいはプロテインGを固相に固定し、固相を血清と反応させてＩｇＧを濃度依存的に捕捉し、更に標識コンカナバリンAまたは標識レンズカリナリスアグルチニンなどの標識レクチンを作用させて、結合した標識物を定量する方法である。しかし、プロテインAやプロテインGはＩｇＧとのみ親和性を示すため、他種の糖タンパク質に関して用いることはできない。

【００１１】また、特開平５－８７８１４公報は、糖鎖の末端にガラクトース残基を含有する糖鎖量の測定方法を提案している。すなわち抗ＩｇＧ抗体の活性フラグメントを固定した固相に、血清を反応させてＩｇＧを濃度依存的に捕捉し、これに標識レクチンを作用させてＩｇＧ糖鎖の末端ガラクトース残基に結合させ、結合した標識物を定量する方法である。この方法は、該特許公開公報の実施例１のリウマチ患者と正常人血清の実測結果（該特許公開公報の図２）が示すように、各測定試料（血清）にて固相に捕捉されるＩｇＧ総量が異なる。すなわち、抗原抗体反応により捕らえられるＩｇＧ量は、測定試料（血清）中のＩｇＧ濃度に依存していることが示されている。この方法にてＩｇＧの全糖鎖量に対する特定糖鎖量の割合を測定するには、該実施例１のごとく、別途何等かの方法にて予めＩｇＧ濃度測定し、各測定試料のＩｇＧ濃度を一定濃度に調製しなくてはならない。

【００１２】これらの方法は、固相上に特定糖タンパク質を濃度依存的に捕捉し、捕捉された特定糖タンパク質の糖鎖にレクチンを介して結合する標識物量を測定する方法であり、測定試料中の特定糖タンパク質濃度が増減すれば結合する標識物量も影響を受ける。よって、臨床診断的に重要となる特定糖タンパク質の特定糖鎖含有割合は、固相に捕捉された特定糖タンパク質の糖鎖全量を別途に測定し、［レクチンの結合した糖鎖量／全糖鎖量］として算出する必要性がある。

【００１３】谷口らは、糖タンパク質の糖鎖の抗体－レクチン酵素免疫測定法に関して報告している（生物物理化学．35巻3 号，第45～50頁，1991年）。この方法は、固相に固定した抗体にて特定糖タンパク質を濃度依存的に捕捉し、これに酵素標識したレクチンを反応させて結合する酵素量を測定する方法であり、特定糖タンパク質の全糖鎖量に対する特定糖鎖量の割合の変化を調べるには、酵素標識した２種以上のレクチンを用いて同一試料を測定し、各レクチンの反応程度を結合した酵素活性の比率として比較する必要があるため、複数のレクチンに対して検量線を別途に作製する等の煩雑な操作を伴う。

【００１４】

【発明が解決しようとする課題】以上に説明したように、薄相クロマトグラフィー、ゲル電気泳動、ガスクロマトグラフィー、液体クロマトグラフィー、アフィニティークロマトグラフィーなどの方法では、特定糖タンパク質の全糖鎖量に対する特定糖鎖量の割合を測定するには操作性がわるく、検体処理時間が極めて長く臨床現場などの検査などにはとても応用できないと言う問題がある。また、レクチンを用いた前述した従来法を応用して特定糖タンパク質の特定糖鎖含有割合を測定しようとすると、これらの方法はいずれも固相上に捕捉される特定糖タンパク質の量が、各測定試料中に含まれる特定糖タンパク質の濃度に応じて変化するため、［レクチンの結合した糖鎖量／全糖鎖量］を知るためには、いちいち固相に捕捉された特定糖タンパク質の糖鎖全量を別途に測定する必要がある。或いは検査対象である試料中の特定糖タンパク質の濃度が常に一定になるように試料の濃度

を調整する必要があり、そのためにはいちいち試料中の特定糖タンパク質の濃度を測定しなければならないと言う問題があった。

【００１５】以上の様に、従来技術では簡便操作にて特定糖タンパク質の全糖鎖量に対する特定糖鎖量の割合を測定することはできない。本発明では、これらの従来法の欠点に鑑み、免疫学的手法とレクチンの親和性を組み合わせ使用し、その度に固相に捕捉された特定糖タンパク質の糖鎖全量を別途に測定したり、或いは検査対象である試料中の特定糖タンパク質の濃度が常に一定になるように試料の濃度を調整するとか、別途試料中の特定糖タンパク質の濃度を測定の度に測定するとか言う手間のかかる操作を改良し、より簡便な操作で特定糖タンパク質の全糖鎖量に対する特定糖鎖量の割合を測定する方法を提供することを目的とするものである。

【００１６】また、そのほか後述する本発明の好ましい態様に於いては、前記目的のほか、それぞれ更に次のような目的を有する。即ち、更に本発明は、よりノイズが少なく、また、測定の幅の広い特定糖タンパク質の全糖鎖量に対する特定糖鎖量の割合を測定する方法を提供することを目的とするものである。

【００１７】更に本発明の別の目的は、病気の診断に有用な特定糖タンパク質の全糖鎖量に対する特定糖鎖量の割合を測定する方法を提供することを目的とするものである。

【００１８】更に本発明の別の目的は、高感度で容易に検出できる特定糖タンパク質の全糖鎖量に対する特定糖鎖量の割合を測定する方法を提供することを目的とするものである。

【００１９】

【課題を解決するための手段】前記課題を達成するために、本発明の糖タンパク質の全糖鎖量に対する特定糖鎖量の割合を測定する方法は次の構成を有するものである。

【００２０】（１）（ａ）特定糖タンパク質のタンパク質部分に特異的親和性を有する抗体および／または該抗体の活性フラグメントの一定量を固定した固相と、前記特定糖タンパク質を含有する試料の液相とを接触させることにより、該固相上の該抗体および／または該抗体の活性フラグメントに該特定糖タンパク質を飽和状態まで捕捉させた後、固相と液相を分離し、次いで分離した固相と該糖タンパク質の特定糖鎖に対して親和性を有する標識化されたレクチンを含有する液相を接触させ、該固相上に捕捉されている特定糖タンパク質の特定糖鎖に標識化されたレクチンを結合させ、次いで固相と液相を分離し、分離した固相にレクチンを介して結合している標識物量を測定し、該標識物量を

（ｂ）一定量の該特定タンパク質が含有する全糖鎖量に対する特定糖鎖の割合が既知である標準試料を用いて前記（ａ）と同様の方法により同様の標識物量の測定を行い、その結果に基いてあらかじめ作成された該標識物量と該特定タンパク質が含有する全糖鎖量に対する特定糖鎖の割合との関係を示す検量値データと比較することにより前記試料に含有されている該特定糖タンパク質の全糖鎖量に対する特定糖鎖割合を決定することを特徴とする特定糖タンパク質の全糖鎖量に対する特定糖鎖量の割合の測定方法。

【００２１】（２）固相に固定した抗体および／または該抗体の活性フラグメントが、測定対象となる特定糖鎖を含まない抗体および／または該抗体の活性フラグメントである前記（１）項に記載の特定糖タンパク質の全糖鎖量に対する特定糖鎖割合の測定方法。

【００２２】（３）特定糖タンパク質を含有する試料が、採取された尿、血液、血漿、血清から選ばれた１種であって、固相に固定化した抗体および／または該抗体の活性フラグメントのすべてに特定糖タンパク質が結合する濃度以上で該特定糖タンパク質を含有する試料である前記（１）項または（２）項に記載の特定糖タンパク質の全糖鎖量に対する特定糖鎖割合の測定方法。

【００２３】（４）標識化されたレクチンが、酵素またはビオチンのいずれかで標識されたレクチンである前記（１）～（３）項のいずれかに記載の特定糖タンパク質の全糖鎖量に対する特定糖鎖量の割合の測定方法。

【００２４】本発明者等は、特定糖タンパク質の全糖鎖量に対する特定糖鎖量の割合を簡便に測定すべく鋭意研究を重ねた結果、まず、測定しようとする特定糖タンパク質のタンパク質部分に特異的親和性を有する一定量の抗体および／または該抗体の活性フラグメント（抗体の抗原認識部位を含む抗体の断片を言う）が固定された固体の担体などの不溶化した固相に、特定糖タンパク質を含有する試料溶液の液相を反応させ、抗原抗体反応を飽和状態まで行うことによって前記特定糖タンパク質の一定量を固相に補捉し、固相と液相を分離し、固相に補捉されられた特定糖タンパク質の特定糖鎖に親和性を有する標識化されたレクチンを反応させ、特定糖鎖残基部分に標識物を結合させ、固相に結合した標識物量を測定し、その標識物量から、あらかじめ特定糖鎖量割合がわかっている標準試料を用いて作成してある標識物量と特定糖鎖の割合との関係を示す検量線などの検量値データを用いて、試料中の特定糖タンパク質の全糖鎖量に対する特定糖鎖量の割合が簡単に測定できることを見い出し前記本発明に至ったものである。

【００２５】通常、糖タンパク質の含有する糖鎖種と数は、糖タンパク質種により一定であるが、疾病によりその糖鎖構造が変化する場合、特定糖タンパク質に含有される全糖鎖数は変化しないが糖鎖種の割合が変化することになる。そこで測定の対象となる特定の糖タンパク質のタンパク質部分に特異的親和性を有する抗体および／またはその活性フラグメントの一定量を固定した固相を用いて測定対象試料中に含まれる特定糖タンパク質と本

発明の抗原抗体反応を飽和状態まで進めると、測定試料（血液、血清等）中より固相上に特定糖タンパク質が一定量捕らえられることになり、この事は、特定糖タンパク質由来の限られた糖鎖種を一定数固相に捕捉することと等しい。そして、この固相に特定糖鎖種を認識して結合する標識レクチンを反応させれば、結合する標識物量は固相に捕捉された全糖鎖数に対する特定糖鎖数、すなわち特定糖鎖割合を反映する事になる。

【００２６】以下、更に具体的に本発明の詳細を説明するが、下記に挙げる具体的物質および物質群などは例示であって、なんらこれのみに限定する事を意味するものではない。

【００２７】本発明で使用する抗体は、その由来を特に限定されるものではなく、ほ乳類等（たとえばマウス、ラット、ウサギなど）に特定糖タンパク質またはその精製物を抗原として投与し、免疫して得られた抗血清、腹水液などをそのまま用いるか、あるいは従来の方法である塩析法、ゲルろ過法、イオン交換クロマトグラフィー、電気泳動法、アフィニティークロマトグラフィーなどで精製してポリクローナル抗体として用いることができる。あるいは、抗原で感作した哺乳類等の抗体産生細胞（脾臓細胞、リンパ節細胞など）とミエローマ細胞とから雑種細胞（ハイブリドーマ）を得て調製したモノクローナル抗体または従来公知の塩析法、各種クロマトグラフィーにより調製したモノクローナル抗体を用いることができる。

【００２８】これらの抗体は抗体分子自体を使用してもよく、また、これらの抗体を酵素処理して得られるＦａｂ、Ｆａｂ’またはＦ（ａｂ’）２といった抗体の活性フラグメント（抗体の抗原認識部位を含む抗体の断片を言う）を使用しても良い。

【００２９】固相の担体形状としては、使用目的に応じた適宜の形状を選定すればよく、たとえば、ビーズ状、テストプレート状、チューブ状、ディスク状、スティック状、ラテックス状などが例示できる。また、その素材としては、通常の酵素免疫測定法（ＥＩＡ）用担体として用いられるもの、たとえば、ガラス、多糖類（セルロース、デキストラン、デンプン、デキストリンなど）またはその誘導体、シリカゲル、多孔性セラミックス、金属酸化物、各種合成樹脂（たとえばプロピレン、塩化ビニル、酢酸ビニル、プロピオン酸ビニル、アクリル酸、アクリル酸エステル、メタクリル酸、メタクリル酸エステル、スチレン、メチルスチレン、ブタジエン、イソプレン、アクリルアミド、アクリロニトリル、メタクリロニトリルなどの重合物もしくは共重合物）、またはこれらに公知の手段によりスルホン基、アミノ基などの反応性官能基を導入したものなどが挙げられる。

【００３０】これらの固相となる担体の形状や大きさは測定対象が同一の糖タンパク質の場合にはできるだけ同一形状で同一の大きさの担体を用いる事が、この各担体の上にそれぞれ同一種類の一定量の前記抗体および／またはその活性フラグメントを固定する場合に極めて操作が楽になるので好ましい。もちろんその大きさや形状が多少異なっても使用可能であるが、その場合にはこれらの各担体の上にそれぞれ同一種類の一定量の前記抗体および／またはその活性フラグメントが固定されるように、これらの抗体の適用量が一定量になるよう配慮すればよい。

【００３１】固相である担体への抗体の固定方法は、物理的吸着法、共有結合法、架橋法等の固定化酵素におけると同様の方法を応用すればよく、たとえば、千畑一郎編「固定化酵素」（昭和５０年３月２０日、（株）講談社発行）第９～７５頁などに記載の公知の手法が応用できる。

【００３２】各担体の上にそれぞれ同一種類の一定量の抗体および／またはその活性フラグメントを固定するには、担体形状が例えばビーズ状、ディスク状、スティック状、ラテックス状など容器としての機能を持たない形状の担体の場合で、それぞれ担体の形状や大きさが同じ担体を用いる場合には、一定濃度の抗体および／またはその活性フラグメントを含む液中に浸してしまって抗体および／またはその活性フラグメントの固定化操作を行う方法が簡便である。しかし、各それぞれの担体に、同一濃度の抗体および／またはその活性フラグメントを含む液を一定量づつ適用する方法を採用してもよい。また、担体形状が例えばテストプレート状、チューブ状など容器としての機能を持つ形状の担体の場合には、通常、各担体に同一濃度の抗体および／またはその活性フラグメントを含む液を一定量づつ入れて固定化操作を行うのが一般的である。しかし、いずれの担体においても、各担体にそれぞれ実質的に同一量の抗体および／またはその活性フラグメントが固定される方法であれば固定化方法はいかなる方法でもよく、何等上記の例示した方法に限られるものではない。

【００３３】固相に固定した抗体および／または該抗体の活性フラグメントは測定対象となる特定糖鎖を含まない抗体および／または該抗体の活性フラグメントであることが測定の際のノイズを少なくでき、測定の幅が広がるので好ましい。ここで測定対象となる特定糖鎖を含まない抗体および／または該抗体の活性フラグメントとは、固相に固定された抗体および／または該抗体の活性フラグメントに含有されている特定糖鎖構造を分解する事により特定糖鎖を含まない抗体および／または該抗体の活性フラグメントとした場合も当然含まれる意味である。

【００３４】固相に固定した抗体および／または該抗体の活性フラグメントに含有されている糖鎖構造を分解する場合は、ノイラミニダーゼやβ－ガラクトシダーゼ等の酵素反応を利用して行うこともできるが、該固相を過ヨウ素酸水溶液に浸すことにより糖鎖構造を酸化分解す

る化学反応が好ましく行われる。

【００３５】レクチンとしては、目的の糖鎖構造を認識して結合するものを用いればよく、たとえば、ヒママメレクチン、ヒイロチャワンタケレクチン、マッシュルームレクチン、コンカナバリンＡ、ドリコスクレクチン、チョウセンアサガオレクチン、レンチルレクチン、ロータスレクチン、イヌエンジュレクチン、インゲンマメレクチン、ピーナッツレクチン、エンドウマメレクチン、アメリカヤマゴボウレクチン、ダイズレクチン、ニホンニワトコレクチン、ハリエニシダレクチン、小麦胚芽レクチンなどが例示される。

【００３６】レクチンと標識物を結合させて、標識化されたレクチンを作製するには、架橋剤として、グルタルアルデヒド、過ヨウ素酸、マレイミド化合物（Ｎ－スクシミジル－２－マレイミドアセテート、Ｎ－スクシニミジル－４－マレイミドプチレート、Ｎ－スクシミジル－４－（Ｎ－マレイミドメチル）－シクロヘキサン－１－カルボキシレート、Ｎ－スルホスクシニミジル－４－（Ｎ－マレイミドメチル）－シクロヘキサン－１－カルボキシレートなど）、ジマレイミド化合物（Ｎ，Ｎ’－オキシジメチレンマレイミド、Ｎ，Ｎ’－ｏ－フェニレンジマレイミドなど）等が使用できる。

【００３７】レクチンを標識化するための標識物としては、酵素（たとえば、β－ガラクトシダーゼ、ペルオキシダーゼ、アルカリフォスファターゼ、グルコースオキシダーゼ、グルコース－６－リン酸デヒドロゲナーゼ等）あるいはビオチンなど何らかの方法で比較的容易に検出可能なものを使用すればよい。そのほか放射性同位元素、補酵素、蛍光色素、化学発光物質などの標識物を用いてもよいが、高感度で容易に検出しやすい点で、酵素あるいはビオチンが特に好ましい。

【００３８】標識物として酵素あるいはビオチンが高感度で特に好ましい理由は、酵素の触媒作用（例えば発色酵素基質を用いた場合、酵素１分子が複数の基質分子に作用し、酵素分子数の数倍の基質を発色させる増幅効果がある。）やビオチンのこの種の標識物として使用する場合の良く知られているＡＢＣ法（アビジン－ビオチン－コンプレックス法）ではビオチンとアビジンの重合物（アビジン１分子はビオチン４分子と結合可能であり、ビオチンの２分子架橋物とアビジンを共存させるとビオチン・アビジン重合体が形成される。例えば酵素標識アビジンを用いて作成したビオチン・アビジン重合体は多数の酵素を含有する。この酵素標識アビジン・ビオチン重合体は、まだ他のビオチンとも結合可能であるので、ビオチンの結合した固相に接触させることにより、固相に結合しているビオチン量と比例した量の酵素標識ビオチン・アビジン重合体が固相に結合する。すなわち固相のビオチン１分子に多数の酵素が結合するので、その酵素の触媒作用を利用する。）の使用が可能であり、酵素量を比較的容易に測定可能な検出系（例えば吸光度測定、蛍光測定、発光測定等）を用いて高感度に検出できるからである。

【００３９】標識化については１９８７年５月１５日医学書院発行　石川榮治、河上忠、宮井潔編集「酵素免疫測定法」第３版　第７５～１５１頁ないしは千畑一郎「固定化酵素」（昭和５０年３月２０日、（株）講談社発行）第９～７５頁などに準じた方法が応用できる。

【００４０】測定対象となる試料（被検体）としては、測定の必要とされる糖タンパク質を含有するものであれば特に限定はなく、たとえば、尿、血液、血漿、血清などの糖タンパク質を含有する生体由来の試料またはその糖タンパク質の精製した糖タンパク質が挙げられる。特に尿、血液、血漿、血清などの糖タンパク質を含有する生体由来の試料を用いる場合には、例えば慢性関節リウマチや癌などの病気の診断に適用できて好ましい。

【００４１】該試料の特定糖タンパク質の全糖鎖量に対する特定糖鎖量の割合を測定するには、固相に固定した特定糖タンパク質のタンパク質部分に特異的親和性を有する一定量の抗体および／または該抗体の活性フラグメントのすべてに特定糖タンパク質が結合する濃度以上で特定糖タンパク質を含有する試料を用いることが肝要である。　尿、血液、血漿、血清などの生体由来の試料などは特定糖タンパク質の含有濃度はほぼ一定の範囲にあるので、一度調査しておけばどの程度の量の抗体および／または該抗体の活性フラグメントが固定された固相を用意しておけば、固相に固定した前記一定量の抗体および／または該抗体の活性フラグメントのすべてに特定糖タンパク質が結合する濃度以上で特定糖タンパク質を含有する試料に相当し、該固相上の該抗体および／または該抗体の活性フラグメントに該特定糖タンパク質を飽和状態まで捕捉させ得る状態とし得るかは容易に判定し得るのである。

【００４２】また、標識化されたレクチンを糖タンパク質の糖鎖に結合させるには、通常ｐＨ６～９の範囲の燐酸緩衝液、トリス－塩酸緩衝液等の溶液として使用する。以下、本発明の理解を容易にするため、図３に本発明法および図４に特開平５－８７８１４公報に記載された方法を簡略モデル図として示し比較する。

【００４３】図３、図４において、１は固相、２は特定糖タンパク質のタンパク質部分に特異的親和性を有する抗体、３は糖タンパク質、４、５はいずれも糖タンパク質の糖鎖を示しているが、４は特定のレクチンと結合し得る特定糖鎖、５は特定のレクチンと結合しない糖鎖、６はレクチン、７は標識物を示している。

【００４４】図３（Ａ）および図４（Ａ）は、固相１に固定した抗体２と、抗原である特定糖タンパク質３の抗原抗体反応を示している。図４（Ａ）に示される特開平５－８７８１４公報の方法では、固相１に固定された抗体２のうち特定糖タンパク質濃度に依存した量が抗体と反応するのに対して、図３（Ａ）に示された本発明方法

では固相１に固定した全ての抗体２と特定糖タンパク質３を反応させる。特定糖タンパク質３と該抗体２の反応は、糖鎖構造の差異に関係なく、特定糖タンパク質３のタンパク質部分を認識して進むので、該抗原抗体反応を飽和状態まで進めることにより固相１上に捕捉される特定糖タンパク質３の全糖鎖量に対する特定糖鎖量の割合は、試料中に存在する特定糖タンパク質の全糖鎖量に対する特定糖鎖量の割合と同じになる。一方、特定糖鎖濃度の測定法である特開平５－８７８１４公報の方法にて本発明法と同様に抗原抗体反応を飽和状態まで行うことは、糖鎖濃度測定可能範囲の上限を越え、測定不可能となることを意味する。

【００４５】なぜならば、この方法は特定糖鎖濃度の測定を目的としているので、固相１に固定された一定数の抗体２が飽和しない範囲で特定糖タンパク質３を抗体２に結合させる必要があるからである。すなわち測定対象試料中の特定糖タンパク質３の濃度が大きい場合には、特定糖タンパク質３が全抗体２の内の全数よりは少ない範囲でかなり多くの抗体２に結合し、試料中の特定糖タンパク質３の濃度が小さい場合には、特定糖タンパク質３が全抗体２の内の全数よりは少ない範囲で前者の場合よりはより少なめの数の抗体２に結合する。こうすることによって試料中の特定糖タンパク質３の濃度の測定ができるが、もし試料中の特定糖タンパク質３の濃度が大きい場合にも小さい場合にも共に固相１に固定された抗体２が飽和する濃度以上の試料を用いた場合には、いずれの場合も特定糖タンパク質３が固相１に固定された抗体２のすべてに結合してしまうため、この両者は同一の濃度であると判定される事になり、従って抗原抗体反応を飽和状態まで行うことは、糖鎖濃度測定可能範囲の上限を越え、糖鎖濃度の測定が不可能となるのである。

【００４６】以上が、既知の特定糖鎖濃度測定法と本発明の方法が異なる第一点である。以上の抗原抗体反応により特定糖タンパク質３を固相１上に捕らえた後、固相と液相を分離することにより、他の共存物と特定糖タンパク質３とを分離することができる。

【００４７】図３（Ｂ）と図４（Ｂ）は固相上に捕らえた特定糖タンパク質３の特定糖鎖４と標識レクチン溶液の液相を接触させる反応を示している。図４（Ｂ）の特開平５－８７８１４公報の方法では、抗原抗体反応にて特定糖タンパク質濃度に依存した量の特定糖タンパク質３が抗体２により固相１上に捕捉されるため、特定糖タンパク質３上の特定糖鎖４に結合するレクチン６も特定糖タンパク質濃度に依存して増減する。一方、図３（Ｂ）に示されている抗原抗体反応を飽和状態まで進める本発明方法では、固相１上に一定量の特定糖タンパク質３を捕えているので、固相１に捕捉した特定糖タンパク質３の特定糖鎖４に結合する標識レクチン量は、特定糖タンパク質一定量当りの特定糖鎖量、すなわち特定糖タンパク質の全糖鎖量に対する特定糖鎖量の割合に比例

する。本発明はこの原理を利用して、特定糖タンパク質の全糖鎖量に対する特定糖鎖量の割合を測定する事を目的としたものである。一方、特開平５－８７８１４公報の方法は、本来、糖鎖濃度測定法であるので、本発明の目的を達成できないのである。

【００４８】また、本発明方法と特開平５－８７８１４公報の方法が異なる更なる点は、測定に際して測定試料と同様の方法で測定される標準物質（標準試料）である。特開平５－８７８１４公報の方法の標準物質は、特定糖鎖濃度既知であれば良いが、本法の標準物質は特定糖タンパク質の全糖鎖量に対する特定糖鎖量の割合が既知でなくてはならない。本法の標準物質の特定糖鎖割合の分析は、成書（新生化学実験講座３糖質Ｉ、糖タンパク質、１９９０年５月２１日発行、東京化学同人）に記載の何れかの方法を用いて行えばよいが、高速液体クロマトグラフィーにより正確な分析を行うことが好ましい。このように標準物質となる特定糖鎖割合の異なる２つ以上、好ましくは３つ以上の試料で歩ければ より好ましいが、かかる複数の標準試料の当該特定糖タンパク質の全糖鎖量に対する特定糖鎖量の割合を上述した高速液体クロマトグラフィーその他の適宜の手段によりあらかじめ分析しておく。このようにして得られた、いくつかの特定糖タンパク質の全糖鎖量に対する特定糖鎖量の割合が既知の標準物質を用い、本発明と同様の手法、すなわち当該特定糖タンパク質のタンパク質部分に特異的親和性を有する抗体および／または該抗体の活性フラグメントの一定量を固定した固相と、前記特定糖タンパク質を含有する特定糖鎖量の割合が既知の標準試料の液相とを接触させることにより、該固相上の該抗体および／または該抗体の活性フラグメントに該特定糖タンパク質を飽和状態まで捕捉させた後、固相と液相を分離し、次いで分離した固相と該糖タンパク質の特定糖鎖に対して親和性を有する標識化されたレクチンを含有する液相を接触させ、該固相上に捕捉されている特定糖タンパク質の特定糖鎖に標識化されたレクチンを結合させ、次いで固相と液相を分離し、分離した固相にレクチンを介して結合している標識物量を測定する。その結果、特定糖鎖量の割合に応じた標識物量（標識物量は例えば吸光度などで調べるので、仮にここでは標識物量を吸光度とこう指標に置き換えて説明する。もちろん標識物の種類に応じて吸光度以外のものが指標になることがある事は当然である。）、すなわち該特定糖タンパク質の特定糖鎖量の割合に応じた吸光度などの検量値データが得られる。この検量値データは、例えば後述する図１に示すような検量線に加工しておく事が便利である。このような検量線、すなわち、吸光度と該特定糖タンパク質の特定糖鎖量の割合との関係をポリグラフをあらかじめ作成しておけば、測定したい試料を前述した本発明方法に従って測定し、その試料の標識物量（例えば吸光度の値）を検出すれば、前記検量線などの検量値データから、容易に該

特定糖タンパク質の全糖鎖量に対する特定糖鎖割合を知ることができるのである。

【００４９】このように検量線などの検量値データさえ作成されていれば、本発明の実質的な操作、すなわち固相上に該特定糖タンパク質を飽和状態まで捕捉させ、固相と液相を分離し、次いで分離した固相と該糖タンパク質の特定糖鎖に対して親和性を有する標識化されたレクチンを含有する液相を接触させ、次いで固相と液相を分離し、分離した固相にレクチンを介して結合している標識物量を測定する操作は比較的短時間でき、従って数多くの試料の測定も容易になり、臨床現場などの短時間で多くの試料の測定を行う必要のある場合に、極めて有用な測定方法を提供できるのである。

【００５０】以上、本発明においては該抗原抗体反応を飽和状態とする反応条件を採用し、更に特定糖タンパク質の全糖鎖量に対する特定糖鎖量の割合が既知の標準物質を用いることによって、初めて特定糖タンパク質の全糖鎖量に対する特定糖鎖量の割合が直接測定できる。

【００５１】

【作用】

（１）本発明は、固相に固定した一定量の抗体および／または該抗体の活性フラグメントのすべてに特定糖タンパク質が結合する濃度以上で特定糖タンパク質を含有する試料を用いることにより、抗原抗体反応が飽和状態まで進み、固相に結合する特定糖タンパク質量は一定となる。通常、糖タンパク質の糖鎖種と数は、糖タンパク質種により定まっており、固相に一定量の特定糖タンパク質を捕捉することは、限られた糖鎖種を一定量捕らえることと等しい。そして、この固相に特定糖鎖種を認識して結合する標識レクチンを反応させれば、結合する標識物量は固相に捕捉された全糖鎖数に対する特定糖鎖数、すなわち特定糖鎖割合を反映する。よって、特定糖タンパク質の糖鎖総量を試料毎に別途測定することなく、特定糖タンパク質の全糖鎖量に対する特定糖鎖量の割合を直接測定することができる。また、かかる操作は前述の特定糖タンパク質の糖鎖総量を試料毎に別途測定すると言う余分な工程を必要とせず、しかも比較的短時間でできるので、より簡便な操作で特定糖タンパク質の全糖鎖量に対する特定糖鎖量の割合を測定する方法を提供できる。

【００５２】（２）また、固相に固定した抗体および／または該抗体の活性フラグメントが、測定対象となる特定糖鎖を含まない抗体および／または該抗体の活性フラグメントである本発明の好ましい態様によれば、よりノイズが少ない特定糖タンパク質の全糖鎖量に対する特定糖鎖割合の測定方法を提供でき、また、測定の幅を広げる事ができる。

【００５３】例えば吸光度計にて吸光度を測定する場合、吸光度計の性能として可能な吸光度測定範囲は機器により定まっている（吸光度０～２．４まで測定可能な機種が一般的である）。本発明の測定試料の吸光度測定値は、測定試料と盲検の吸光度差として求めるので、盲検の吸光度（ノイズ）が小さいほど測定試料の吸光度の計測可能な範囲が広くなる。（例えばノイズの吸光度が２．０である場合、吸光度０～２．４が測定可能な吸光度計を用いて測定できる吸光度範囲は、２．０～２．４となる。これに対して、ノイズの吸光度が０．１である場合の測定可能な吸光度範囲は、０．１～２．４の範囲となる。また、特定糖鎖割合１％当りの吸光度変化量を一定とした場合、同一吸光度計を用いて測定可能な特定糖鎖割合の範囲は、ノイズの吸光度が小さい方が広くなる。つまり、特定糖鎖割合の高い試料まで測定できる。）

（３）また、特定糖タンパク質を含有する試料が、採取された尿、血液、血漿、血清から選ばれた１種であって、固相に固定化した抗体または該抗体の活性フラグメントのすべてに特定糖タンパク質が結合する濃度以上で該特定糖タンパク質を含有する試料である本発明の好ましい態様によれば、これらの試料は、患者の試料採取時の特定糖鎖割合を反映するので、健常人の標準的な特定糖鎖割合と比べてその割合の増減により容易に病気の診断に適用する事ができる特定糖タンパク質の全糖鎖量に対する特定糖鎖割合の測定方法を提供できる。

【００５４】（４）また、標識化されたレクチンが、酵素またはビオチンのいずれかで標識されたレクチンである本発明の好ましい態様によれば、前述した酵素の触媒作用などによる増幅効果などにより、高感度でより容易に検出できる特定糖タンパク質の全糖鎖量に対する特定糖鎖量の割合の測定方法を提供できる。

【００５５】

【実施例】以下に本発明の理解を容易にするため、実施例を挙げて説明するが、本発明はこの実施例のみに限定されるものではない。

【００５６】実施例１

（ＩｇＣ糖鎖割合標準物質の作製）市販のヒトＩｇＣを0.1M 酢酸緩衝液（pH 5）にて溶解（10 mg/ml）しノイラミニダーゼを加えて３７℃、６５時間放置後、凍結保存する（試料１）［レクチンが結合可能な特定糖鎖を有する特定糖タンパク質］。試料１の一部を融解し、β－ガラクトシダーゼを加えて３７℃、４８時間放置する（試料２）［試料１のレクチンが結合可能な特定糖鎖を酵素でダイジェストして得たものであり、レクチンが結合可能な特定糖鎖の含有量が試料１より少ない特定糖タンパク質］。試料１及び２を硫酸アンモニウムの分画沈澱法により精製し、添加した酵素を除去する。試料１及び２のタンパク質濃度を紫外光（２８０ｎｍ）吸収法により定量し、同一に調製する。タンパク質濃度を合わせた試料２に対して試料１を２５、５０、７５（v/v%）で混合し、混合試料を作製した。

【００５７】試料１及び２の糖鎖分析は、高速液体クロ

マトグラフィーにより行う。すなわち、成書（髙橋禮子編著「糖蛋白質糖鎖研究法」平成元年９月１日発行　学会出版センター出版）の高速液体クロマトグラフィー（ＨＰＬＣ）により特定糖鎖割合を測定した。詳細には、試料１及び２を各々をヒドラジン分解して糖鎖を切り出し、２－アミノピリジンで標識後、逆相クロマトグラフィーにて分析し、クロマトグラムより全糖鎖量に対する糖鎖末端に２個のガラクトースを含有する糖鎖（特定糖鎖）の割合を算出した。試料１及び２をＨＰＬＣにて糖鎖分析した結果、全糖鎖量に対する該糖鎖の割合は、それぞれ３１．７％及び７．０％であった。この試料１及び２を標準試料とした。

【００５８】実施例２

（抗ヒトＩｇＧ抗体固定マイクロプレートの作製）ヤギ抗ヒトＩｇＧ抗体を生理食塩水に溶解し（10μg/ml）、多数の同じ形で同じ大きさのウェルを有するマイクロプレートの各ウェルに100 μｌづつ分注した。これを４℃、２４時間放置後、精製水にて洗浄し、１(w/v%)ウシ血清アルブミン（ＢＳＡ）水溶液を300 μｌづつ分注して３時間室温放置した。放置後、マイクロプレートを精製水にて洗浄し、0.1(w/v%) 過ヨウ素酸ナトリウム含有50mMクエン酸緩衝液（pH 4）を200 μｌづつ分注し、４℃、３０分間静置し、精製水により洗浄した後測定に用＊いた。

【００５９】実施例３

（マイクロプレートへのＩｇＧ捕捉量の確認）実施例２で作製した抗ヒトＩｇＧ抗体固定マイクロプレートに、試料希釈用液［0.05(v/v%)ポリオキシエチレンソルビタンモノラウレートを含む10mMリン酸緩衝液 pH7.4］50μｌを分注し、ヒト血清を生理食塩水で１／１，１／２，１／４倍希釈した測定試料を各々20μｌ添加し、室温で１時間振盪した。マイクロプレートを生理食塩水で３回洗浄した後、特定糖タンパク質（この場合はヒトＩｇＧ）のタンパク質部分を認識して結合するペルオキシダーゼ標識抗ヒトＩｇＧ抗体溶液（0.2μg/ml、試料希釈用液で希釈）を各々50μｌ添加し、１時間反応させた。生理食塩水にて５回洗浄後、固相に結合しているペルオキシダーゼの酵素活性を測定するために、ｏ－フェニレンジアミン（1mg/ml）および過酸化水素水［0.015(v/v%)］を含むリン酸－クエン酸緩衝液（pH 4.8）を50μｌ添加し、２０分間室温で反応させた。２Ｎ硫酸の50μｌを加えて反応を停止し、４９２ｎｍの吸光度を測定した。各測定試料の吸光度測定値を表１に示す。

【００６０】

【表１】

血清希釈	ブランク	１／１	１／２	１／４
Ａ492nm	0．１２５	0．７０８	0．６７３	0．７０９

【００６１】表１の各吸光度測定値はどの場合もほぼ同様である。すなわち、試料中のヒトＩｇＧ濃度が変化してもどの場合も同様にマイクロプレート上に一定量のヒトＩｇＧが捕捉されていることを示している。

【００６２】これは、抗原抗体反応を飽和状態まで進めた結果であり、抗原抗体反応が１時間以内に飽和状態になることが確認できた。また、このような予備実験により、試料が固相に固定化した抗体および／または該抗体の活性フラグメントのすべてに特定糖タンパク質が結合する濃度以上で該特定糖タンパク質を含有する試料であるかどうか、容易に判定出来、一度判定したらその後はどの試料に対してはどの程度の量の抗体および／または該抗体の活性フラグメントが固定された固相を用い、どの程度の時間反応させたらよいかの関係が明確に樹立される事になる。

【００６３】すなわち、例えば臨床検査で血清を試料とする場合には、どの固相を用いてどの程度の反応時間を行えば、抗原抗体反応を飽和状態まで進めることができるかを明白にする事ができる。

【００６４】実施例４

（糖鎖末端に２個のガラクトースを含有する糖鎖の割合変化に伴う吸光度測定値の変動）実施例２で作成したマイクロプレートに、試料稀釈用液50μｌを分注し、これに実施例１で調製した各混合試料と試料１及び２のそれぞれを別々に20μｌ添加した後、室温で１時間振盪した。生理食塩水にて３回洗浄後、ベルオキシダーゼ標識ヒママメレクチン１２０［（株）ホーネンコーポレーション製］の0.1μg/ml溶液［0.05(V/V%)ポリオキシエチレンソルビタンモノラウレート含有１０ｍＭリン酸緩衝液（pH7.4）］を 50μｌ分注し、１時間反応させた。以後実施例３と同様な方法で操作しマイクロプレート用の吸光度計を用いて吸光度を測定した。尚、盲検は測定試料に代えて生理食塩水を用いて並行して同様操作することにより行った。

【００６５】表２は、各試料の吸光度測定結果、実施例１にて確認した試料１及び２の特定糖鎖割合［表２の（）内］、及び各混合試料の特定糖鎖割合の理論値［試料１及び２を混合した後の特定糖鎖割合を計算により算出した値であり、例えば試料２に対して試料１を２５(V/V%)混合した場合、7.0（試料２の特定糖鎖割合）×0.75（試料２の混合比率）＋31.7（試料１の特定糖鎖割合）×0.25（試料１の混合比率）＝13.2となる。］を示している。

【００６６】

【表２】

17　　18

	盲検	試料１	試料２	試料２に対する試料１の混合割合		
				２５	５０	７５
A492nm	0.112	0.438	0.166	0.238	0.301	0.356
ΔA492nm	－	0.326	0.054	0.126	0.189	0.244
特定糖鎖割合理論値(%)		(31.7)	(7.0)	13.2	19.4	25.5

【００６７】注1：（）内は、実施例１の高速液体クロマトグラフィーでの測定値。
注2：ΔA492nmは、各試料と盲検の吸光度（A492nm）の差を示す。
表２に示した試料１及び２の吸光度測定値（ΔA492nm）と実施例１で確認した特定糖鎖割合の関係を図１に示す。図１は実質上、全糖鎖量に対する特定糖鎖（この場合は糖鎖末端に２個のガラクトースを含有する糖鎖）の割合と吸光度の関係を示す検量線を示す事になる。
【００６８】表２に示した試料１及び２の吸光度測定値（ΔA492nm）と特異糖鎖割合は良好な直線関係を示した。すなわち図１に示した直線は、ヒトＩｇＧの特定糖鎖割合（この場合は、ヒトＩｇＧの全糖鎖量に対する糖＊鎖末端に２個のガラクトースを含有する糖鎖の割合）が31.7%まで直線的に吸光度変化する事を示している。この検量線を用いれば、特定糖鎖割合未知の測定試料の吸光度測定値（ΔA492nm）より特定糖鎖割合を確定することができるし、例えば表２に示した混合試料の吸光度測定値（ΔA492nm）より特定糖鎖割合を算出することもできる。
【００６９】表３は、図１を用いて表２に示した各混合試料の吸光度測定値（ΔA492nm）から特定糖鎖割合に変換した結果である。
【００７０】
【表３】

	試料２に対する試料１の混合割合(V/V%)		
	２５	５０	７５
検量線より算出した特定糖鎖割合（%）	12.5	18.7	24.3

【００７１】表３に示した各混合試料の特定糖鎖割合は、表２に示した該当混合試料の特定糖鎖割合と近似値を示すことから、図１の様な検量線を用いて各混合試料の吸光度測定値（ΔA492nm）より導き出した特定糖化割合は正しいと言える。
【００７２】したがって図１に示したような検量線を検量値データとして保有している場合には、同様の糖タンパク質を含有する試料について同様の測定をし、得られた吸光度の値から図１の検量線を使用する事により当該試料中の全糖鎖量に対する特定糖鎖（この場合は糖鎖末端に２個のガラクトースを含有する糖鎖）の割合がただちにわかる事になる。
【００７３】実施例５
（健常人血清ＩｇＧとＲＡ患者血清ＩｇＧとの測定値の差異）健常人血清４検体およびＲＡ患者血清３０検体を実施例４と同様に操作して測定した結果、図２に示す様に健常人血清とＲＡ患者血清の吸光度測定値の分布は、明らかに異なり、ＲＡ患者血清の吸光度測定値の方が分布として低い傾向を示した。図２は健常人血清４検体およびＲＡ患者血清３０検体のそれぞれのＩｇＧの実施例４と同様の操作によって測定した各検体（試料）の吸光度を示す図である。
【００７４】検体測定と並行して実施例４の特定糖鎖割合既知の標準試料（試料１及び２）を測定し、検量線を作成して使用すれば、当該検体中の全糖鎖量に対する特定糖鎖（この場合は糖鎖末端に２個のガラクトースを含有する糖鎖）の割合がただちに判明する。
【００７５】尚、図２の結果は、ＲＡ患者において血清中のＩｇＧの全糖鎖量に対する特定糖鎖量（糖鎖末端に２個のガラクトースを含有する糖鎖）の割合が健常人よりも低下していることを示している。
【００７６】実施例６
（ヒトトランスフェリンの特定糖鎖割合の測定）ヒトトランスフェリンを 0.1M 酢酸緩衝液（pH 5）に溶解後、フコシダーゼを添加し、３７℃、２４時間反応させた。反応終了後、トランスフェリンを精製して脱フコース処理試料（レクチンが結合する特定糖鎖がほとんど存在しない試料）とした。この試料と未酵素処理（レクチンが結合する特定糖鎖が存在する）のヒトトランスフェリン溶液のタンパク質濃度を合わせた後、各種割合にて混合して測定試料を調製した。
【００７７】一方、ヤギ抗ヒトトランスフェリン抗体固定マイクロプレートを実施例２と同様の方法で作成し、実施例４と同様の方法で各測定試料を反応させた。生理食塩水にて３回洗浄後、アルカリフォスファターゼ標識ヒイロチャワンタケレクチンを反応させ、生理食塩水にて５回洗浄後、アルカリフォスファターゼの活性を成書（１９８７年５月１５日医学書院発行　石川榮治、河上

患、宮井潔編集「酵素免疫測定法」第３版　第５８頁）に従って測定した。その結果、測定試料の未酵素処理試料割合が増すに従って実施例４と同様、吸光度測定値が直線的に増加した。これは、ヒイロチャワンタケレクチンがフコース含有糖鎖を認識して結合する結果であり、吸光度測定値はフコース含有糖鎖割合に従って変化することを確認した。

【００７８】実施例７

（α－フェトプロテインの特定糖鎖割合の測定）α－フェトプロテインを 0.1M 酢酸緩衝液（pH 5）に溶解後、フコシダーゼを添加し、３７℃、２４時間反応させた。反応終了後、α－フェトプロテインを精製して脱フコース処理試料（レクチンが結合する特定糖鎖がほとんど存在しない試料）とした。この試料と未酵素処理の溶液（レクチンが結合する特定糖鎖が存在する）のタンパク質濃度を合わせた後、各種割合にて混合して測定試料を調製した。

【００７９】一方、ヤギ抗α－フェトプロテイン抗体固定マイクロプレートを実施例２と同様の方法で作成し、実施例４と同様の方法で各測定試料を反応させた。生理食塩水にて３回洗浄後、ビオチン標識レンチルレクチンを室温にて１時間反応させ、生理食塩水にて５回洗浄した。更にペルオキシダーゼ標識アビジンを室温にて２０分間反応させ、生理食塩水にて５回洗浄後、実施例３と同様の方法で吸光度測定した。その結果、測定試料の未酵素処理試料割合が増すに従って実施例４と同様、吸光度測定値が直線的に増加した。これは、レンチルレクチンがフコース含有糖鎖を認識して結合する結果であり、吸光度測定値はフコース含有糖鎖割合に従って変化した。

【００８０】

【発明の効果】

（１）本発明は、特定糖タンパク質の糖鎖総量を別途測定する必要がなく、試料中の特定糖タンパク質の全糖鎖量に対する特定糖鎖量の割合を簡便かつ迅速に測定することができる特定糖鎖量の割合の測定方法を提供できる。

【００８１】本発明によってはじめて、直接的に特定糖タンパク質の全糖鎖量に対する特定糖鎖量の割合を簡便かつ迅速に測定できる方法が提供されるのである。

（２）また、固相に固定した抗体および／または該抗体の活性フラグメントが、測定対象となる特定糖鎖を含まない抗体および／または該抗体の活性フラグメントである本発明の好ましい態様とすることにより、よりノイズが少なく、また、測定の幅の広い特定糖タンパク質の全糖鎖量に対する特定糖鎖割合の測定方法が提供できる。

【００８２】（３）また、特定糖タンパク質を含有する試料が、採取された尿、血液、血漿、血清から選ばれた１種であって、固相に固定化した抗体および／または該抗体の活性フラグメントのすべてに特定糖タンパク質が結合する濃度以上で該特定糖タンパク質を含有する試料である本発明の好ましい態様とすることにより、容易に病気の診断に適用する事ができる特定糖タンパク質の全糖鎖量に対する特定糖鎖割合の測定方法を提供できる。

【００８３】（４）また、標識化されたレクチンが、酵素またはビオチンのいずれかで標識されたレクチンである本発明の好ましい態様とすることにより、高感度で検出の容易な特定糖タンパク質の全糖鎖量に対する特定糖鎖量の割合の測定方法が提供できる。

【図面の簡単な説明】

【図１】本発明の一実施例における吸光度と特定タンパク質の特定糖鎖との関係を示した図。

【図２】縦軸を吸光度として健常人血清４検体とＲＡ患者血清３０検体を本発明で測定した結果を示した図。

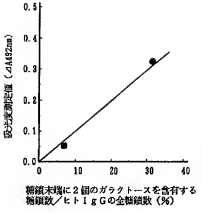
【図３】本発明の測定法のための反応工程の一部をモデル的に示した簡略モデル図。

【図４】特開平５－８７８１４号記載の方法の説明のための反応工程の一部をモデル的に示した簡略モデル図。

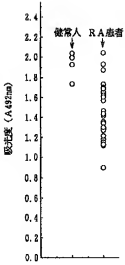
【符号の説明】

１　固相
２　抗体
３　糖タンパク質
４　特定のレクチンと結合し得る特定糖鎖
５　特定のレクチンと結合しない糖鎖
６　レクチン
７　標識物

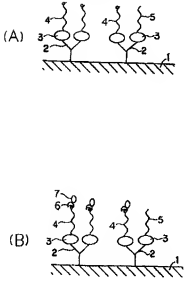
【図１】


吸光度測定値（λ492nm）
0.5
0.4
0.3
0.2
0.1
0.0
0
10
20
30
40
糖鎖末端に２個のガラクトースを含有する
糖鎖数／ヒトＩｇＧの全糖鎖数（％）

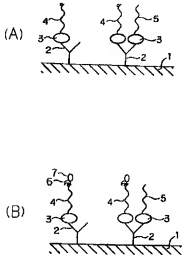
【図２】


健常人
RA患者
吸光度（λ492nm）
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0

【図３】


(A)
(B)

【図４】


(A)
(B)

CLAIMS

[Claim(s)]
[Claim 1]The liquid phase containing labeled lectin characterized by comprising the following is contacted, Lectin labeled by specific sugar chain of specific glycoprotein caught on this solid phase is combined, Subsequently, the amount of marker substances which separated the solid phase and the liquid phase and has been combined with the separated solid phase via lectin is measured, A rate of a specific sugar chain over the total amount of sugar chains in which this specific protein of the (b) constant rate contains this amount of marker substances measures the same amount of marker substances by the same method as the above (a) using a standard sample which is known, By comparing with measuring value data in which a relation with a rate of a specific sugar chain over this amount of marker substances beforehand created based on the result and the total amount of sugar chains which this specific protein contains is shown. A measuring method of a rate of the amount of specific sugar chains over the total amount of sugar chains of specific glycoprotein determining a specific sugar chain rate over the total amount of sugar chains of this specific glycoprotein contained in the aforementioned sample.
(a) The solid phase which fixed to a protein portion of specific glycoprotein a constant rate of an active fragment of an antibody and/or this antibody which has specific compatibility.
It is compatibility to a specific sugar chain of the solid phase which separated the solid phase and the liquid phase and was subsequently separated after making an active fragment of this antibody on this solid phase, and/or this antibody catch this specific glycoprotein to saturation by contacting the liquid phase of a sample containing said specific glycoprotein, and this glycoprotein.

[Claim 2]A measuring method of a specific sugar chain rate over the total amount of sugar chains of the specific glycoprotein according to claim 1 which is an active fragment of an antibody and/or this antibody in which an active fragment of an antibody fixed to the solid phase and/or this antibody does not contain a specific sugar chain used as a measuring object.
[Claim 3]A sample containing specific glycoprotein is one sort chosen from urine, blood, plasma, and a blood serum which were extracted, A measuring method of a specific sugar chain rate over the total amount of sugar chains of the specific glycoprotein according to claim 1 or 2 which is a sample which contains this specific glycoprotein above concentration which specific glycoprotein combines with all the active fragments of an antibody fixed in the solid phase, and/or this antibody.
[Claim 4]A measuring method of a rate of the amount of specific sugar chains over the total amount of sugar chains of the specific glycoprotein according to any one of claims 1 to 3 whose labeled lectin is the lectin by which the sign was carried out with either an enzyme or biotin.

[Translation done.]

DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Industrial Application]This invention relates to the measuring method of the rate of the amount of specific sugar chains over the total amount of sugar chains of specific glycoprotein.
[0002]
[Description of the Prior Art]Glycoprotein is the naturally-ocurring polymers which a sugar chain which makes a constituent protein which makes amino acid a constituent, and sugar combined.
The great portion of protein which exists in the living body is glycoprotein.
Sugar, such as galactose, N-acetyl glucosamine, mannose, and fucose, connected the sugar chain of glycoprotein branched, and, as for the sugar chain structure, it has played the important role which serves as a mark of each substance on the occasion of a biosynthesis in the living body and metabolic turnover. The relation between the sugar chain structure of this glycoprotein and a disease is studied in detail, the appearance of the glycoprotein which has sugar chain structure which is different from a healthy person in cancer or rheumatoid arthritis comes to be reported, and even if it faces a clinical diagnosis, sugar chain structure analysis is becoming important.
Hereafter, the sugar chain structure change accompanying the actual condition and the disease of a clinical diagnosis is explained in detail by making rheumatoid arthritis into an example.
[0003]Rheumatoid arthritis (it abbreviates to RA below) is a disease which is mainly concerned with arthritis of frequent occurrence nature.
It is a kind of an autoimmune disease which shows an inflammatory change of collagenous tissue.
Diagnosis of RA is mainly performed to this in the clinical feature by considering the amount of rheumatoid factors (it abbreviates to RF below) as an immunological factor (Toshikazu Hirose, medicine, and pharmaceutical sciences 30 volumes, 33-page 1993).
[0004]It is known that RF is an antibody which recognizes some parts of human immunoglobulin G (it abbreviates to IgG below) as an antigen, For RF measurement, the reagent and patient's serum which were made to adsorb Homo sapiens IgG at latex are mixed, The reagent and patient's serum which made the singer PUROTTSU (Singer-Plotz) method who investigates an agglutination reaction, and the human immunoglobulin stick to a sheep red blood cell are mixed, and the WARA rose (Waaler-Rose) method for seeing cohesiveness, etc. are used. However, the singularity of RF existence in RA patient is lower than RF exists in other disease patients, such as liver disease and a cancer patient, at high frequency and being checked by 3 to 7% of healthy persons. Therefore, RF measured as an index of RA diagnosis at the clinical spot has not necessarily been a high diagnostic index of singularity at RA.
[0005]Recently, research was done in detail about the sugar chain which exists in IgG in a blood serum, and it was reported that the abundance of IgG top sugar chain end galactose residue is decreasing intentionally by RA patient (R. B. Parekh et al. Nature 316 452 (1985)). That is, it was reported that the sugar chain end galactose residue of IgG was increasing the rate that dyad is missing even to about 50% by RA patient to being about 25% of all the sugar chains for a healthy person. And when sugar chain end galactose residue suffers a loss, antigenicity appears in IgG and leading RF is expected. As for the deficit of this sugar chain end galactose, it is pointed out that it is not the secondary-like phenomenon that the medication of RA became a cause and produced but a specific phenomenon which happens to RA patient universally. (**** Koichi edit "glycoconjugate" protein nucleic acid enzyme special issue the 1924 - 1962 page Heisei 3(1991) August 10 issue KYORITSU SHUPPAN Co., Ltd.).
Therefore, development of the measuring method is expected noting that detection of the sugar chain end galactose deficiency of IgG has a possibility of becoming a new index of RA diagnosis.
[0006]As mentioned above, when a close relation can be found out to sugar chain structure change of symptoms and glycoprotein, development of a determination method is furthered noting that the measured value of the rate of the amount of specific sugar chains over the total amount of sugar chains of specific glycoprotein has a possibility of becoming a useful index in clinical judgement.
[0007]As a method of measuring the sugar chain of glycoprotein, Thin phase chromatography, gel electrophoresis,

gas chromatography, Although there are liquid chromatography, affinity chromatography, etc. (the quality I of new chemical experiment lecture trisaccharide, glycoprotein, May 21, 1990 issue, Tokyo Kagaku Dojin) and it is widely used for the sugar chain analysis of glycoprotein, If sensitometry, operativity, sample processing time, etc. are taken into consideration, it is difficult to use these methods as clinical examination method as they are. The measuring method using lectin was able to be considered as a method of solving this problem. Lectin is protein which recognizes specific sugar chain structure and in which specific compatibility is shown.
Sugar chain recognition ability is explained in a compendium (the quality I of new chemical experiment lecture trisaccharide, glycoprotein, May 21, 1990 issue, Tokyo Kagaku Dojin, the 9-18th page) in detail.

[0008]Although the sandwich technique of the antibody-lectin which a JP,61-20867,A gazette catches specific glycoprotein on a concentration dependence target by the antibody which has specific compatibility in target glycoprotein, and detects the amount of sugar chains of the caught specific glycoprotein by sign lectin is reported, As a reference standard is known from carrying out dilution preparation with buffer solution by preparation of standard urine for working-example 1.V., this method is the method of measuring the amount of sugar residues depending on antigen concentration.
It is not the method of measuring a rate of the amount of specific sugar chains over the total amount of sugar chains of an antigen.

[0009]Although the JP,4-130274,A gazette caught target glycoprotein on the concentration dependence target by lectin with the sugar chain of specific glycoprotein, and compatibility and the lectin lectin sandwich technique detected by the sign lectin which has the target sugar chain and compatibility of glycoprotein further is proposed, The reaction of lectin has low singularity as compared with an antigen-antibody reaction, and it is impossible to measure correctly the rate of the amount of specific sugar chains over the total amount of sugar chains of specific glycoprotein.
[0010]The JP,3-73857,A gazette has proposed the method of measuring the amount of IgG in which the end galactose residue of the sugar chain suffered a loss about the specific sugar chain concentration measurement method using RA-related lectin. This method is the method of quantifying the marker substance which fixed protein A or the protein G to the solid phase, made the solid phase react to a blood serum, and caught IgG on the concentration dependence target, and also sign lectins, such as sign concanavalin A or sign lens KARINARISU agglutinin, were made to act, and was combined. However, since protein A and the protein G show IgG and compatibility, they cannot be used about glycoprotein of other type.
[0011]The JP,5-87814,A gazette has proposed the measuring method of the amount of sugar chains which contains galactose residue at the end of a sugar chain. That is, it is the method of quantifying the marker substance which made the blood serum react, caught IgG on the concentration dependence target, made sign lectin act on this, made the solid phase which fixed the active fragment of the anti-IgG antibody combine with the end galactose residue of an IgG sugar chain, and was combined with it. As the sufferer from rheumatism of the working example 1 of this patent published unexamined application and the measured result (drawing 2 of this patent published unexamined application) of a normal people blood serum show this method, the IgG total amounts caught by the solid phase in each test portion (blood serum) differ. That is, depending on the IgG concentration in a test portion (blood serum) for the amount of IgG caught by an antigen-antibody reaction is shown. In order to measure the rate of the amount of specific sugar chains over the total amount of sugar chains of IgG by this method, like this working example 1, IgG density measurement must be separately carried out beforehand by a certain method, and the IgG concentration of each test portion must be prepared to fixed concentration.
[0012]These methods are the methods of measuring the amount of marker substances which catches specific glycoprotein on a concentration dependence target on the solid phase, and is combined with a sugar chain of caught specific glycoprotein via lectin.
The amount of marker substances which will be combined if specific glycoprotein concentration in a test portion fluctuates is also affected by influence.
Therefore, the specific sugar chain content ratio of the specific glycoprotein which becomes important in clinical diagnosis measures separately the sugar chain whole quantity of the specific glycoprotein caught by the solid phase, and there is the necessity of computing as [the amount of sugar chains / a total amount of sugar chains which lectin combined].
[0013]Taniguchi and others has reported the antibody-lectin enzyme immunoassay of the sugar chain of glycoprotein (living thing physical chemistry .35 volume 3 an item, the 45-50th page, 1991). This method is the method of measuring the amount of enzymes which catch specific glycoprotein on a concentration dependence target in an antibody fixed to the solid phase, and lectin which carried out enzyme labeling to this is made to react, and is combined.
In order to investigate change of the rate of the amount of specific sugar chains over the total amount of sugar chains of specific glycoprotein, The same sample is measured using two or more sorts of lectins which carried out

enzyme labeling, and since it is necessary to compare as a ratio of enzyme activity which combined a reaction grade of each lectin, it is accompanied by complicated operation of producing an analytical curve separately to two or more lectins.

[0014]

[Problem to be solved by the invention]As explained above, in methods, such as thin phase chromatography, gel electrophoresis, gas chromatography, liquid chromatography, and affinity chromatography, Operativity is bad for measuring the rate of the amount of specific sugar chains over the total amount of sugar chains of specific glycoprotein, and there is a problem which sample processing time says very long is inapplicable to the inspection of the clinical spot etc. at all in it. If the conventional method using lectin mentioned above tends to be applied and it is going to measure the specific sugar chain content ratio of specific glycoprotein, Since each of these methods changes according to the concentration of the specific glycoprotein in which the quantity of the specific glycoprotein caught on the solid phase is contained in each test portion, In order to know [the amount of sugar chains / the total amount of sugar chains which lectin combined], it is necessary to measure separately the sugar chain whole quantity of the specific glycoprotein caught by the solid phase one by one. Or the concentration of the sample needed to be adjusted so that the concentration of specific glycoprotein in the sample which is a subject of examination might always become fixed, and there was a problem referred to as having to measure the concentration of specific glycoprotein in a sample one by one for that purpose.

[0015]As mentioned above, in conventional technology, the rate of the amount of specific sugar chains over the total amount of sugar chains of specific glycoprotein cannot be measured by simple operation. In this invention, the compatibility of the immunological technique and lectin is combined and used in view of the fault of these conventional methods, Measure separately the sugar chain whole quantity of the specific glycoprotein caught by the solid phase at every time, or, Or adjust the concentration of a sample or so that the concentration of specific glycoprotein in the sample which is a subject of examination may always become fixed. The time-consuming operation referred to as measuring the concentration of specific glycoprotein in a sample to the degree of measurement separately is improved, and it aims at providing the method of measuring the rate of the amount of specific sugar chains over the total amount of sugar chains of specific glycoprotein by simpler operation.

[0016]In addition, in the desirable mode of this invention mentioned later, it has the respectively still more nearly following purposes besides the aforementioned purpose. Namely, this invention aims more to let a noise provide the method of measuring the rate of the amount of specific sugar chains over the total amount of sugar chains of wide specific glycoprotein of measurement few.

[0017]Another purpose of this invention aims at providing the method of measuring the rate of the amount of specific sugar chains over the total amount of sugar chains of useful specific glycoprotein to sick diagnosis.

[0018]Another purpose of this invention aims at providing the method of measuring the rate of the amount of specific sugar chains over the total amount of sugar chains of specific glycoprotein easily detectable by high sensitivity.

[0019]

[Means for solving problem]In order to attain the aforementioned problem, the method of measuring the rate of the amount of specific sugar chains over the total amount of sugar chains of glycoprotein of this invention has the next composition.

[0020](1) by contacting the solid phase which fixed to the protein portion of (a) specific glycoprotein the constant rate of the active fragment of an antibody and/or this antibody which has specific compatibility, and the liquid phase of the sample containing the above-mentioned specific glycoprotein, After making the active fragment of this antibody on this solid phase, and/or this antibody catch this specific glycoprotein to saturation, The solid phase which separated the solid phase and the liquid phase and was subsequently separated, and the liquid phase containing the labeled lectin which has compatibility to the specific sugar chain of this glycoprotein are contacted, The lectin labeled by the specific sugar chain of the specific glycoprotein caught on this solid phase is combined, Subsequently, the amount of marker substances which separated the solid phase and the liquid phase and has been combined with the separated solid phase via lectin is measured, The rate of a specific sugar chain over the total amount of sugar chains in which this specific protein of the (b) constant rate contains this amount of marker substances measures the same amount of marker substances by the same method as the above (a) using the standard sample which is known, By comparing with the measuring value data in which a relation with the rate of a specific sugar chain over this amount of marker substances beforehand created based on the result and the total amount of sugar chains which this specific protein contains is shown. A measuring method of the rate of the amount of specific sugar chains over the total amount of sugar chains of the specific glycoprotein determining the specific sugar chain rate over the total amount of sugar chains of this specific glycoprotein contained in the aforementioned sample.

[0021](2) A measuring method of the specific sugar chain rate over the total amount of sugar chains of specific glycoprotein given in the aforementioned (1) clause which is an active fragment of an antibody and/or this antibody

in which the active fragment of the antibody fixed to the solid phase and/or this antibody does not contain the specific sugar chain used as a measuring object.

[0022](3) The urine, blood, plasma in which the sample containing specific glycoprotein was extracted, It is one sort chosen from the blood serum, A measuring method of the specific sugar chain rate over the total amount of sugar chains of specific glycoprotein given in the aforementioned (1) clause or (2) clauses which are a sample which contains this specific glycoprotein above the concentration which specific glycoprotein combines with all the active fragments of the antibody fixed in the solid phase, and/or this antibody.

[0023](4) A measuring method of the rate of the amount of specific sugar chains over the total amount of sugar chains of specific glycoprotein given in either of the aforementioned (1) - (3) clauses whose labeled lectins are the lectin by which the sign was carried out with either an enzyme or biotin.

[0024]The result of having repeated research wholeheartedly this invention persons measuring the rate of the amount of specific sugar chains over the total amount of sugar chains of specific glycoprotein simple, To first, the solid phase which the carrier etc. of the solid in which the active fragment (the fragment of the antibody containing the antigen recognition site of an antibody is said) of a constant rate of antibodies and/or this antibody which has specific compatibility was fixed to the protein portion of the specific glycoprotein which it is going to measure insolubilized, Make the liquid phase of the sample solution containing specific glycoprotein react, and the constant rate of the above-mentioned specific glycoprotein is caught to the solid phase by performing an antigen-antibody reaction to saturation, Separate the solid phase and the liquid phase and the labeled lectin which has compatibility is made to react to the specific sugar chain of the specific glycoprotein caught by the solid phase. The amount of marker substances which was made to combine a marker substance with a specific sugar chain residue portion, and was combined with the solid phase is measured, Measuring value data, such as an analytical curve which shows the relation between the amount of marker substances currently created from the amount of marker substances using the standard sample which the amount rate of specific sugar chains understands beforehand, and the rate of a specific sugar chain, is used, It finds out that the rate of the amount of specific sugar chains over the total amount of sugar chains of specific glycoprotein in a sample can measure easily, and results in aforementioned this invention.

[0025]Usually, although the total number of sugar chains which the sugar chain kind and number which glycoprotein contains contain in specific glycoprotein with a glycoprotein kind when the sugar chain structure changes with illnesses, although it is fixed does not change, the rate of a sugar chain kind will change. Then, if the antigen-antibody reaction of the specific glycoprotein contained in a measuring object sample using the solid phase which fixed the constant rate of the antibody which has specific compatibility, and/or its active fragment to the protein portion of specific glycoprotein which is the target of measurement, and this invention is advanced to saturation, A fixed quantity of specific glycoprotein will be caught on the solid phase from the inside of test portions (blood, a blood serum, etc.), and this thing is equal to catching the sugar chain kind in which the specific glycoprotein origin was restricted to a fixed number of solid phases. And if the sign lectin which recognizes a specific sugar chain kind to this solid phase, and is combined with it is made to react, the amount of marker substances to combine will reflect the number of specific sugar chains to the total number of sugar chains caught by the solid phase, i.e., a specific sugar chain rate.

[0026]The next concrete substance, substance group, etc. are illustration, and the following does not mean limiting only to this at all, although the details of this invention are explained concretely.

[0027]The antibody in particular used by this invention is not a thing which has the origin limited, The mammals (for example, a mouse, Latt, USAKI, etc.) etc. are medicated with specific glycoprotein or its refining thing as an antigen, A curing salting method, a gel-filtration method, ion exchange chromatography, an electrophoresis method, affinity chromatography, etc. which are the conventional methods can refine using antiserum, ascites liquid, etc. which were produced by carrying out immunity as it is, and it can use as a polyclonal antibody. Or a monoclonal antibody or a conventionally publicly known curing salting method for having obtained and prepared the hybrid cell (hybridoma) with the antigen from antibody forming cells (a spleen cell, a lymph node cell, etc.), such as the mammals which carried out sensitization, and a myeloma cell, The monoclonal antibody prepared with various chromatography can be used.

[0028]These antibodies may use the antibody molecule itself, and may use the active fragment (the fragment of the antibody containing the antigen recognition site of an antibody is said) of antibodies, such as Fab and Fab' which are produced by carrying out enzyme treatment of these antibodies, or F(ab') 2.

[0029]What is necessary is just to select the proper form according to the purpose of use as carrier form of the solid phase, and the shape of a bead, the shape of a test plate, tube shape, the shape of a disk, stick shape, the shape of latex, etc. can be illustrated. What is used as a usual carrier for enzyme immunoassay (EIA) as the raw material, for example, glass and polysaccharide (cellulose, dextran, and starch ---) The derivatives, such as dextrin, silica gel, porous ceramics, a metallic oxide and various synthetic resins (for example, propylene, VCM/PVC, and vinyl acetate ---) Vinyl propionate, acrylic acid, acrylic ester, methacrylic acid, Methacrylic acid ester, styrene, methylstyrene, butadiene, isoprene, Polymer, such as acrylamide, acrylonitrile, and a methacrylonitrile, copolymer,

or the thing that introduced reactive functional groups, such as a sulfone group and an amino group, into these by the publicly known means is mentioned.

[0030]Using the carrier of the same size with identical shape as much as possible, when a measuring object is the same glycoprotein the form and the size of a carrier used as these solid phases, Since operation becomes easy extremely when it fixes a constant rate of the aforementioned antibodies of an identical kind, and/or the active fragment of those on each of this carrier, respectively, it is desirable. What is necessary is for it to be usable even if the size and form differ from each other somewhat, of course, but just to consider so that a constant rate of the aforementioned antibodies of an identical kind and/or the active fragment of those may be fixed on each of these carriers in that case, respectively, and the dosage of these antibodies may turn into a constant rate.

[0031]Also in immobilized enzyme, such as a physical adsorption process, a covalent binding procedure, and a cross-linking method, the fixing method of the antibody to the carrier which is the solid phase should just apply the same method, for example, can apply the publicly known technique of a description to the 9-75th pages of 1000 Hata [Ichiro] editing "immobilized enzyme" (Showa 50(1975) March 20, Kodansha Issue) etc.

[0032]In order to fix a constant rate of antibodies of an identical kind, and/or the active fragment of those on each carrier, respectively, Carrier form for example, in the case of the carrier of form without the function as containers, such as the shape of a bead, the shape of a disk, stick shape, and the shape of latex, When using the carrier with respectively same form and size of a carrier, the method of dipping into the liquid containing the antibody of fixed concentration and/or its active fragment, and performing fixed operation of an antibody and/or its active fragment is simple. however -- each --- the method of applying a fixed quantity of liquid which contains the antibody of the same concentration and/or its active fragment in each carrier every may be adopted. In the case of the carrier of the form in which carrier form has a function as containers, such as the shape for example, of a test plate, and tube shape, it is common to usually put in a fixed quantity of liquid which contains the antibody of the same concentration and/or its active fragment in each carrier every, and to perform fixed operation. However, in which carrier, if it is a method by which the same quantity of an antibody and/or its active fragment are substantially fixed to each carrier, respectively, what kind of method may be sufficient as a fixing method, and it will not be restricted to how to have illustrated the above at all.

[0033]Since that it is an active fragment of an antibody and/or this antibody which does not contain the specific sugar chain used as a measuring object can lessen the noise in the case of measurement and the width of measurement spreads, the active fragment of the antibody fixed to the solid phase and/or this antibody is preferred. The active fragment of the antibody which does not contain the specific sugar chain which serves as a measuring object here, and/or this antibody, Also when it is considered as the active fragment of the antibody which does not contain a specific sugar chain by decomposing the specific sugar chain structure contained in the active fragment of the antibody fixed to the solid phase, and/or this antibody, and/or this antibody, it is a meaning naturally included.

[0034]When decomposing the sugar chain structure contained in the active fragment of the antibody fixed to the solid phase, and/or this antibody, can also carry out using enzyme reactions, such as neuraminidase and **-galactosidase, but. The chemical reaction which carries out oxidative degradation of the sugar chain structure is preferably performed by dipping this solid phase in periodic acid solution.

[0035]What is necessary is just to use as lectin what recognizes the target sugar chain structure and is combined, For example, HIMAMAME lectin, HIIRO cup-fungus lectin, mushroom lectin, Concanavalin A, DORIKOSUKU lectin, jimson weed lectin, Lentile lectin, lotus lectin, dog Japanese pagoda tree lectin, kidney-beans lectin, peanut lectin, Pisum sativum lectin, pokeweed lectin, soybean lectin, the Japan sambucus lectin, whin lectin, wheat germ lectin, etc. are illustrated.

[0036]In order to produce the lectin which made combine lectin and a marker substance and was labeled, as a cross linking agent --- glutaraldehyde, periodic acid, and a maleimide compound (N-SUKUSHI midge roux 2-maleimide acetate ---) N-succinimidyl 4-maleimide butyrate, N-SUKUSHI midge roux 4 -(N-maleimide methyl)- Cyclohexane-1-carboxylate, N-sulphosuccinimidyl 4 -(N-maleimide methyl)- Cyclohexane-1-carboxylate etc. can use dimaleimido compounds (N,N'-oxydi methylenemaleimide, N, and N'-o-phenylenedimaleimide etc.) etc.

[0037]As a marker substance for labeling lectin, What is necessary is just to use a detectable thing comparatively easily by some methods, such as enzymes (for example, **-galactosidase, peroxidase, alkaline phosphatase, glucose oxidase, glucose 6-phosphate dehydrogenase, etc.) or biotin. In addition, although marker substances, such as radioisotope, a coenzyme, a fluorochrome, and a chemiluminescence substance, may be used, an enzyme or especially biotin is preferred at the point which is easy to detect by high sensitivity easily.

[0038]An enzyme or biotin especially a desirable Reason by high sensitivity as a marker substance, The catalysis of an enzyme (for example, when a coloring enzyme substrate is used, one molecule of enzymes act on two or more substrate molecules, and) there is the amplification effect in which a several times as many substrate as the number of enzyme molecules is made to color -- the ABC method (the avidin biotin complex method) which it is [in the case of using it as this kind of biotin of a marker substance] good, and is known --- the polymer (biotin tetrad and combination are possible for one molecule of avidin, and) of biotin and avidin If the dyad bridge

construction thing and avidin of biotin are made to live together, a biotin avidin polymer will be formed. For example, the biotin avidin polymer created using enzyme-labeling avidin contains many enzymes. Since it is still combinable with other biotin, the enzyme-labeling biotin avidin polymer of the amount of biotin combined with the solid phase and a proportional quantity combines this enzyme-labeling avidin biotin polymer with the solid phase by making the solid phase which biotin combined contact. That is, since many enzymes combine with one molecule of biotin of the solid phase, the catalysis of the enzyme is used. It is because it can be used and the amount of enzymes can be detected to high sensitivity using measurable detection systems (for example, spectrometry, fluorometry, luminescence measurement, etc.) comparatively easily.

[0039]About labeling, it is May 15, 1987 Igaku-Shoin issue. Eiji Ishikawa, Tadashi Kawakami, the 3rd edition of Kiyoshi Miyai edit "enzyme immunoassay" The method according to the 75-151st page, the 9-75th pages of 1000 Ichiro Hata "immobilized enzyme" (Showa 50(1975) March 20, Kodansha Issue), etc. is applicable.

[0040]The solution which the sample of the living body origin which limitation in particular will not have if the glycoprotein for which measurement is needed is contained as a sample (analyte) used as a measuring object, for example, contains glycoprotein, such as urine, blood, plasma, and a blood serum, or its glycoprotein refined is mentioned. When using the sample of the living body origin which contains glycoprotein, such as urine, blood, plasma, and a blood serum, especially, it can apply, for example to sick diagnosis of rheumatoid arthritis, cancer, etc., and is desirable.

[0041]In order to measure the rate of the amount of specific sugar chains over the total amount of sugar chains of specific glycoprotein of this sample, It is important to use the sample which contains specific glycoprotein above the concentration which specific glycoprotein combines with all the active fragments of a constant rate of antibodies and/or this antibody that have specific compatibility into the protein portion of the specific glycoprotein fixed to the solid phase. Since samples of the living body origin of urine, blood, plasma, a blood serum, etc. have the content concentration of specific glycoprotein in the almost fixed range, If the solid phase to which the active fragment of the antibody of the quantity of how much and/or this antibody was fixed once it investigated is prepared, Are equivalent to the sample which contains specific glycoprotein above the concentration which specific glycoprotein combines with all the active fragments of a constant rate of the aforementioned antibodies, and/or this antibody fixed to the solid phase, Whether it can change into the state where the active fragment of this antibody on this solid phase and/or this antibody may be made to catch this specific glycoprotein to saturation can judge easily.

[0042]In order to combine the labeled lectin with the sugar chain of glycoprotein, it is usually used as solutions, such as a phosphate buffer of the range of pH 6-9, and tris-chloride buffer solution. Hereafter, in order to make an understanding of this invention easy, the method indicated to this invention method and drawing 4 at the JP,5-87814,A gazette is shown in drawing 3 as a simple model figure, and is compared with it.

[0043]Although the antibody to which 1 has the solid phase and 2 has specific compatibility in into the protein portion of specific glycoprotein, and 3 show four in drawing 3 and drawing 4 and each of glycoprotein and 5 shows the sugar chain of glycoprotein, The sugar chain which is not combined with lectin with specific specific sugar chain which can be combined with lectin with specific 4 and 5, and 6 show lectin, and 7 shows the marker substance.

[0044]Drawing 3 (A) and drawing 4 (A) show the antigen-antibody reaction of the antibody 2 fixed to the solid phase 1, and the specific glycoprotein 3 which is antigens. All the antibodies 2 and specific glycoprotein 3 which were fixed to the solid phase 1 are made to react by this invention method shown in drawing 3 (A) by the method of the JP,5-87814,A gazette shown in drawing 4 (A) to the quantity for which it depended on specific glycoprotein concentration among the antibodies 2 fixed to the solid phase 1 reacting to an antibody. Since the reaction of the specific glycoprotein 3 and this antibody 2 recognizes the protein portion of the specific glycoprotein 3 and progresses regardless of the difference in sugar chain structure, The rate of the amount of specific sugar chains over the total amount of sugar chains of the specific glycoprotein 3 caught on the solid phase 1 becomes the same as the rate of the amount of specific sugar chains over the total amount of sugar chains of the specific glycoprotein which exists in a sample by advancing this antigen-antibody reaction to saturation. On the other hand, performing an antigen-antibody reaction to saturation like this invention method by the method of the JP,5-87814,A gazette which is a measuring method of specific sugar chain concentration exceeds the maximum of the sugar chain density measurement possible range, and it means that measurement becomes impossible.

[0045]It is because this method aims at measurement of specific sugar chain concentration, so it is necessary to combine the specific glycoprotein 3 with the antibody 2 in the range with which a fixed number of antibodies 2 fixed to the solid phase 1 are not saturated. Namely, when the concentration of the specific glycoprotein 3 in a measuring object sample is large, The specific glycoprotein 3 combines with quite many antibodies 2 in the range less than the total of Uchi of all the antibodies 2, and when the concentration of the specific glycoprotein 3 in a sample is small, the specific glycoprotein 3 combines with a number smaller than the case of the former of antibodies 2 in the range less than the total of Uchi of all the antibodies 2. Although measurement of the concentration of the specific glycoprotein 3 in a sample can be performed by carrying out like this, When the

sample more than the concentration with which the antibody 2 fixed to the solid phase 1 also when [both] the concentration of the specific glycoprotein 3 in a sample was large and it was small is saturated is used, In order that the specific glycoprotein 3 may combine with all the antibodies 2 fixed to the solid phase 1 in any case, It being judged with these both being the same concentration, therefore performing an antigen-antibody reaction to saturation exceeds the maximum of the sugar chain density measurement possible range, and the measurement of sugar chain concentration of it becomes impossible.

[0046]The above is the first point from which a known specific sugar chain density measuring method and the method of this invention differ. After catching the specific glycoprotein 3 on the solid phase 1 by the above antigen-antibody reaction, other concomitants and specific glycoprotein 3 are separable by separating the solid phase and the liquid phase.

[0047]Drawing 3 (B) and drawing 4 (B) show the reaction for which the specific sugar chain 4 of the specific glycoprotein 3 and the liquid phase of a sign lectin solution which were caught on the solid phase are contacted. In the method of the JP,5-87814,A gazette of drawing 4 (B), Since the specific glycoprotein 3 of quantity for which it depended on specific glycoprotein concentration in the antigen-antibody reaction is caught by the antibody 2 on the solid phase 1, the lectin 6 combined with the specific sugar chain 4 on the specific glycoprotein 3 is also fluctuated depending on specific glycoprotein concentration. On the other hand by this invention method advanced to saturation, the antigen-antibody reaction shown in drawing 3 (B), Since a constant rate of specific glycoprotein 3 is caught on the solid phase 1, the sign lectin content combined with the specific sugar chain 4 of the specific glycoprotein 3 caught to the solid phase 1 is proportional to the rate of the amount of specific sugar chains over the amount of specific sugar chains of sugar chains per specific glycoprotein constant rate, i.e., the total amount of specific glycoprotein. An object using this principle of this invention is to measure the rate of the amount of specific sugar chains over the total amount of sugar chains of specific glycoprotein. On the other hand, originally, since the method of a JP,5-87814,A gazette is a sugar chain density measuring method, it cannot attain the purpose of this invention.

[0048]The further point that this invention method differs from the method of a JP,5-87814,A gazette is a standard substance (standard sample) measured by the same method as a test portion when measuring. Although the standard substance of the method of a JP,5-87814,A gazette should just be specific sugar chain concentration known, the rate of the amount of specific sugar chains of as opposed to the total amount of sugar chains of specific glycoprotein in the standard substance of this method must be known. Although what is necessary is just to perform analysis of the specific sugar chain rate of the standard substance of this method to a compendium (the quality I of new chemical experiment lecture trisaccharide, glycoprotein, May 21, 1990 issue, Tokyo Kagaku Dojin) using which method of a description, it is preferred to conduct exact analysis with high performance chromatography. thus, the specific sugar chain rates used as a standard substance differ — it being more desirable if large, but by three or more samples, preferably, two or more. The means which mentioned above the rate of the amount of specific sugar chains over the total amount of sugar chains of the specific glycoprotein of two or more of these standard samples concerned being high performance chromatography and by which others are proper analyzes beforehand. Thus, the acquired rate of the amount of specific sugar chains over the total amount of sugar chains of some specific glycoprotein uses a known standard substance, The solid phase which fixed to the same technique as this invention, i.e., the protein portion of the specific glycoprotein concerned, the constant rate of the active fragment of an antibody and/or this antibody which has specific compatibility, When the rate of the amount of specific sugar chains containing the above-mentioned specific glycoprotein contacts the liquid phase of a known standard sample, After making the active fragment of this antibody on this solid phase, and/or this antibody catch this specific glycoprotein to saturation, The solid phase which separated the solid phase and the liquid phase and was subsequently separated, and the liquid phase containing the labeled lectin which has compatibility to the specific sugar chain of this glycoprotein are contacted, The amount of marker substances which was made to combine the lectin labeled by the specific sugar chain of the specific glycoprotein caught on this solid phase, subsequently separated the solid phase and the liquid phase and has been combined with the separated solid phase via lectin is measured. As a result, the amount of marker substances according to the rate of the amount of specific sugar chains (since the amount of marker substances is investigated with an absorbance etc., temporarily, the amount of marker substances is transposed to the index called absorbance, and is explained here.) Of course according to the kind of marker substance, naturally, things other than an absorbance may become an index. That is, measuring value data according to the rate of the amount of specific sugar chains of this specific glycoprotein, such as an absorbance, is obtained. As for this measuring value data, it is convenient to process an analytical curve as shown in drawing 1 mentioned later, for example. If the graph which shows the relation of such an analytical curve, i.e., an absorbance and the rate of the amount of specific sugar chains of this specific glycoprotein, is created beforehand, If it measures in accordance with this invention method which mentioned the sample to measure above and the amount of marker substances of the sample (for example, value of an absorbance) is detected, the specific sugar chain rate over the total amount of sugar chains of this specific glycoprotein can be easily known from measuring value data, such as the aforementioned analytical curve.

[0049]If even measuring value data, such as an analytical curve, is created, thus, substantial operation of this invention, Namely, on the solid phase, make this specific glycoprotein catch to saturation, and the solid phase and the liquid phase are separated, Subsequently, the separated solid phase and the liquid phase containing the labeled lectin which has compatibility to the specific sugar chain of this glycoprotein are contacted, Subsequently, operation which measures the amount of marker substances which separated the solid phase and the liquid phase and has been combined with the separated solid phase via lectin can be performed comparatively for a short time, Therefore, when measurement of many samples also needs to become easy and needs to measure many samples in a short time of the clinical spot etc., a very useful measuring method can be provided.
[0050]As mentioned above, the rate of the amount of specific sugar chains over the total amount of sugar chains of specific glycoprotein will not be able to be measured directly without adopting the reaction condition which makes this antigen-antibody reaction saturation in this invention, and also the rate of the amount of specific sugar chains over the total amount of sugar chains of specific glycoprotein using a known standard substance.
[0051]
[Function]
(1) Use a sample which contains specific glycoprotein above concentration which specific glycoprotein combines with all the active fragments of a constant rate of antibodies, and/or this antibody fixed to the solid phase in this invention.
Therefore, an antigen-antibody reaction progresses to saturation and the amount of specific glycoprotein combined with the solid phase becomes fixed.
Usually, a sugar chain kind and number of glycoprotein have become settled with a glycoprotein kind.
It is equal to catching a fixed quantity of limited sugar chain kinds to catch a constant rate of specific glycoprotein to the solid phase.
And if the sign lectin which recognizes a specific sugar chain kind to this solid phase, and is combined with it is made to react, the amount of marker substances to combine will reflect the number of specific sugar chains to the total number of sugar chains caught by the solid phase, i.e., a specific sugar chain rate. Therefore, the rate of the amount of specific sugar chains over the total amount of sugar chains of specific glycoprotein can be measured directly, without measuring the sugar chain total amount of specific glycoprotein separately for every sample. Since the excessive process referred to as that this operation measures separately the sugar chain total amount of the above-mentioned specific glycoprotein for every sample is not needed but it can moreover do comparatively for a short time, the method of measuring the rate of the amount of specific sugar chains over the total amount of sugar chains of specific glycoprotein by simpler operation can be provided.
[0052](2) According to the desirable mode of this invention which is an active fragment of an antibody and/or this antibody which does not contain the specific sugar chain used as a measuring object, the active fragment of the antibody fixed to the solid phase and/or this antibody. The measuring method of the specific sugar chain rate over the total amount of sugar chains of specific glycoprotein with few noises can be provided, and the width of measurement can be expanded.
[0053]For example, when measuring an absorbance with an absorbance meter, the spectrometry range possible as performance of an absorbance meter has become settled by apparatus (a model measurable to the absorbances 0-2.4 is common). Since the spectrometry value of the test portion of this invention is calculated as absorption difference of a test portion and a blank test, the range which can measure the absorbance of a test portion, so that the absorbance (noise) of a blank test is small becomes large. (For example, when the absorbance of a noise is 2.0, the absorbance range which the absorbances 0-2.4 can measure using a measurable absorbance meter is set to 2.0-2.4.) On the other hand, the measurable absorbance range in case the absorbance of a noise is 0.1 turns into the range of 0.1-2.4. When the amount of absorbance variations per 1% of specific sugar chain rate is set constant, as for the range of a measurable specific sugar chain rate, the one where the absorbance of a noise is smaller becomes large using the same absorbance meter. That is, it can measure to a sample with a high specific sugar chain rate.
(3) Urine, blood in which the sample containing specific glycoprotein was extracted, According to the desirable mode of this invention which is one sort chosen from plasma and a blood serum, and is a sample which contains this specific glycoprotein above the concentration which specific glycoprotein combines with all the active fragments of the antibody fixed in the solid phase, or this antibody. Since these samples reflect the specific sugar chain rate at the time of a patient's sampling, they can provide the measuring method of the specific sugar chain rate over the total amount of sugar chains of specific glycoprotein easily applicable to sick diagnosis by the change in the rate compared with healthy persons' standard specific sugar chain percentage.
[0054](4) According to the desirable mode of this invention which is the lectin by which the sign was carried out with either an enzyme or biotin the labeled lectin. By the amplification effect by the catalysis etc. of the enzyme mentioned above, etc., the measuring method of the rate of the amount of specific sugar chains over the total amount of sugar chains of the specific glycoprotein which can be more easily detected by high sensitivity can be provided.

[0055]
[Working example]In order to make an understanding of this invention easy below, an working example is given and described, but this invention is not limited only to this working example.
[0056]Homo sapiens IgG of working-example 1 (production of an IgG sugar chain rate standard substance) marketing is dissolved with 0.1M acetic acid buffer solution (pH 5) (10 mg/ml), neuraminidase is added, and cryopreservation is carried out after 37 ** and 65-hour neglect [the specific glycoprotein which has a specific sugar chain which can combine lectin]. (sample 1) A part of sample 1 is dissolved, **-galactosidase is added, and 37 ** is neglected for 48 hours [(sample 2) the specific glycoprotein with less content of the specific sugar chain which can combine lectin than the sample 1 which digests and obtains with an enzyme the specific sugar chain which can combine the lectin of the sample 1]. The samples 1 and 2 are refined with the fractionation precipitation method of ammonium sulfate, and the added enzyme is removed. The protein concentration of the samples 1 and 2 is quantified with an ultraviolet radiation (280 nm) absorption process, and is prepared identically. The sample 1 was mixed by 25, 50, and 75 (v/v %) to the sample 2 which doubled protein concentration, and the mixed sample was produced.
[0057]Sugar chain analysis of the samples 1 and 2 is conducted with high performance chromatography. That is, the specific sugar chain rate was measured with the high performance chromatography (HPLC) of the compendium (Reiko Takahashi written and edited "glycoprotein sugar chain approach" Heisei 1(1989) September 1 issue Japan Scientific Societies Press publication). In detail, hydrazinolysis of each was carried out for the samples 1 and 2, the sugar chain was cut down, reversed phase chromatography analyzed after the sign by 2-aminopyridine, and the rate of the sugar chain (specific sugar chain) which contains two galactose at the sugar chain end to the total amount of sugar chains from chromatogram was computed. As a result of conducting sugar chain analysis of the samples 1 and 2 in HPLC, the percentages of this sugar chain over the total amount of sugar chains were 31.7% and 7.0%, respectively. These samples 1 and 2 were made into the standard sample.
[0058]The working-example 2 (production of anti-human IgG antibody fixed microplate) goat anti-human IgG antibody was dissolved in the physiological saline (10 microg/(ml)), and it dispensed every [l / 100mu] to each well of the microplate which has a well of the same size in many same forms. Purified water washed this after 4 ** and 24-hour neglect, 1 (w/v %) bovine-serum-albumin (BSA) solution was dispensed every [l / 300mu], and room temperature neglect was carried out for 3 hours. Purified water washed the microplate after neglect, 0.1 (w/v %) sodium-periodate content 50mM citrate buffer solution (pH 4) was dispensed every [l / 200mu], and was settled for 30 minutes 4 **, and after purified water washed, it used for measurement.
[0059]To the anti-human IgG antibody fixed microplate produced in working-example 3 (check of the amount of IgG prehension to a microplate) working example 2, Liquid [10containing polyoxyethylene sorbitan monolaurate mM 0.05 (v/v %) Phosphate buffer solution 7.4 [pH]] 50microfor sample dilution l was dispensed, 20microl addition made respectively 1/1, 1/2, and the test portion diluted 1/4 time with the physiological saline, and human serum was shaken at the room temperature for 1 hour. After a physiological saline washed a microplate 3 times, 50microl addition made respectively the peroxidase-labeling anti-human IgG antibody solution (it dilutes with 0.2 microg [ml] /and the liquid for sample dilution) which recognizes the protein portion of specific glycoprotein (in this case, Homo sapiens IgG), and is combined, and it was made to react for 1 hour. In order to measure the enzyme activity of the peroxidase combined with the solid phase after 5 times washing with the physiological saline, 50microl addition of the phosphoric acid-citrate buffer solution (pH 4.8) containing o-phenylenediamine (1mg/(ml)) and hydrogen peroxide solution [0.015 (v/v %)] was done, and it was made to react at a room temperature for 20 minutes. 50microl of 2N sulfuric acid was added, the reaction was suspended, and the absorbance of 492 nm was measured. The spectrometry value of each test portion is shown in Table 1.
[0060]
[Table 1]

血清希釈	ブランク	1／1	1／2	1／4
A492nm	0．125	0．708	0．673	0．709

[0061]In any case, each spectrometry value of Table 1 is almost the same. That is, even if the Homo sapiens IgG concentration in a sample changes, in any case, it is shown that a constant rate of Homo sapiens IgG is similarly caught on a microplate.
[0062]This is the result of advancing an antigen-antibody reaction to saturation.
It has checked that an antigen-antibody reaction was saturated within 1 hour.
. [whether it is a sample which contains this specific glycoprotein above the concentration which specific glycoprotein combines with all the active fragments of the antibody which the sample fixed in the solid phase, and/or this antibody by such preliminary experiment, and] It can judge easily, and once it judges, to which sample, that relation in which how much should just carry out a time reaction will be clearly established using the solid phase to which the active fragment of the antibody of the quantity of how much and/or this antibody was fixed

after that.

[0063]That is, if it performs how much reaction time using which solid phase in making a blood serum into a sample, for example by a clinical laboratory test, it can be clarified whether an antigen-antibody reaction can be advanced to saturation.

[0064]To the microplate created in working-example 4 (change of the spectrometry value accompanying rate change of the sugar chain which contains two galactose at the sugar chain end) working example 2, Liquid 50mul for sample dilution was dispensed, and after 20microl addition making separate each of each mixed sample and the samples 1 and 2 which were prepared in working example 1 to this, it shook at the room temperature for 1 hour. With a physiological saline, after 3 times washing, 50microl of 0.1 microg [/ml] solutions [0.05 (V/V %) polyoxyethylene-sorbitan-monolaurate content 10mM phosphate buffer solution (pH 7.4)] of the peroxidase-labeling HIMAMAME lectin 120 [HONEN Corporation Make] were dispensed I times, and were made to react for 1 hour. It was operated by the same method as the working example 3 after that, and the absorbance was measured using the absorbance meter for microplates. The blank test was performed by replacing with a test portion and operating it in a similar manner in parallel using a physiological saline.

[0065]Table 2 is the value which computed a specific sugar chain rate after mixing the theoretical-value [samples 1 and 2 of a spectrometry result of each sample, a specific sugar chain rate [inside of () of Table 2] of the samples 1 and 2 checked in working example 1, and a specific sugar chain rate of each mixed sample by calculation. For example, it is set to 7.0(specific sugar chain rate of sample 2)x0.75(mixing ratio of sample 2)+31.7(specific sugar chain rate of sample 1)x0.25(mixing ratio of sample 1)=13.2 when 25 (V/V %) mixing of the sample 1 is carried out to the sample 2.

] is shown.

[0066]

[Table 2]

	盲検	試料1	試料2	試料2に対する試料1の混合割合		
				25	50	75
A492nm	0.112	0.438	0.166	0.238	0.301	0.356
ΔA492nm	–	0.326	0.054	0.126	0.189	0.244
特定糖鎖割合理論値(%)		(31.7)	(7.0)	13.2	19.4	25.5

[0067]Notes 1: The inside of () is the measured value in the high performance chromatography of the working example 1.

Notes 2: deltaA492 nm of differences of the absorbance (A492nm) of each sample and a blank test are shown.

The relation of the specific sugar chain rate checked in the spectrometry value (deltaA492 nm) and the working example 1 of the samples 1 and 2 shown in Table 2 is shown in drawing 1. Drawing 1 will show on parenchyma the analytical curve which shows the rate of a specific sugar chain (in this case, sugar chain which contains two galactose at the sugar chain end) and the relation of an absorbance to the total amount of sugar chains.

[0068]The spectrometry value (deltaA492 nm) and unique sugar chain rate of the samples 1 and 2 which were shown in Table 2 showed the good straight-line relation. That is, it is shown that the specific sugar chain rate (in this case, the sugar chain which contains two galactose at the sugar chain end to the total amount of sugar chains of Homo sapiens IgG comparatively) of Homo sapiens IgG carries out the absorbance variation of the straight line shown in drawing 1 linearly to 31.7%. If this analytical curve is used, a specific sugar chain rate can be decided from the spectrometry value (deltaA492 nm) of the test portion of specific sugar chain rate strangeness, and a specific sugar chain rate can also be computed from the spectrometry value (deltaA492 nm) of the mixed sample shown, for example in Table 2.

[0069]Table 3 is the result of changing into a specific sugar chain rate from the spectrometry value (deltaA492 nm) of each mixed sample shown in Table 2 using drawing 1.

[0070]

[Table 3]

	試料2に対する試料1の混合割合(V/V%)		
	25	50	75
検量線より算出した特定糖鎖割合 (%)	12.5	18.7	24.3

[0071]since the specific sugar chain rate of each mixed sample shown in Table 3 showed the specific sugar chain rate and approximate value of the applicable mixed sample which were shown in Table 2, it was drawn from the spectrometry value (deltaA492 nm) of each mixed sample using an analytical curve like drawing 1 — specific — saccharification — a rate can be said to be the right.

[0072]Therefore, when the analytical curve as shown in drawing 1 is held as measuring value data, By using the analytical curve of drawing 1 from the value of the absorbance obtained by carrying out same measurement about the sample containing the same glycoprotein will show immediately the rate of a specific sugar chain (in this case, sugar chain which contains two galactose at the sugar chain end) over the total amount of sugar chains in the sample concerned.
[0073]The result of having operated working-example 5 (difference in measured value of healthy person blood serum IgG and RA patient's-serum IgG) healthy-person blood serum 4 sample, and RA patient's-serum 30 sample like the working example 4, and having measured them, As shown in drawing 2, distribution of the spectrometry value of a healthy person blood serum and RA patient's serum differed clearly, and the direction of the spectrometry value of RA patient's serum showed the tendency low as distribution. Drawing 2 is a figure showing the absorbance of each sample (sample) measured by the same operation as the working example 4 of each IgG of healthy person blood serum 4 sample and RA patient's-serum 30 sample.
[0074]In parallel to specimen measurement, the standard sample of specific sugar chain rate known of the working example 4 (samples 1 and 2) is measured, and if an analytical curve is prepared and used, the rate of a specific sugar chain (in this case, sugar chain which contains two galactose at the sugar chain end) over the total amount of sugar chains in the sample concerned will become clear immediately.
[0075]The result of drawing 2 shows that the rate of the amount of specific sugar chains (sugar chain which contains two galactose at the sugar chain end) over the total amount of sugar chains of IgG in a blood serum is falling rather than the healthy person in RA patient.
[0076]Fucosidase was added after dissolving working-example 6 (measurement of specific sugar chain rate of Homo sapiens transferrin) Homo sapiens transferrin in 0.1M acetic acid buffer solution (pH 5), and 37 ** was made to react for 24 hours. After ending reaction, transferrin was refined and it was considered as the defucose processing sample (sample in which the specific sugar chain which lectin combines hardly exists). After doubling the protein concentration of the Homo sapiens transferrin solution of this sample and non-enzyme treatment (the specific sugar chain which lectin combines exists), in some numbers, it was comparatively alike, mixed, and the test portion was prepared.
[0077]On the other hand, the goat anti human transferrin antibody fixed microplate was created by the same method as the working example 2, and each test portion was made to react by the same method as the working example 4. Alkaline phosphatase sign HIIRO cup-fungus lectin is made to react after 3 times washing with a physiological saline. The activity of alkaline phosphatase was measured after 5 times washing according to the compendium (with May 15, 1987 Igaku-Shoin issue Eiji Ishikawa, Tadashi Kawakami, and a Kiyoshi Miyai edit "enzyme immunoassay" of edition [3rd] the 58th page) with the physiological saline. As a result, the spectrometry value increased linearly like the working example 4 as the non-enzyme treatment sample rate of the test portion increased. This is the result of HIIRO cup-fungus lectin's recognizing a fucose containing sugar chain, and joining together.
It checked that a spectrometry value changed according to a fucose containing sugar chain rate.

[0078]Fucosidase was added after dissolving working-example 7 (measurement of specific sugar chain rate of alpha fetoprotein) alpha fetoprotein in 0.1M acetic acid buffer solution (pH 5), and 37 ** was made to react for 24 hours. After ending reaction, alpha fetoprotein was refined and it was considered as the defucose processing sample (sample in which the specific sugar chain which lectin combines hardly exists). After doubling the protein concentration of the solution (the specific sugar chain which lectin combines exists) of this sample and non-enzyme treatment, in some numbers, it was comparatively alike, mixed, and the test portion was prepared.
[0079]On the other hand, the goat anti-alpha-fetoprotein antibody fixation microplate was created by the same method as the working example 2, and each test portion was made to react by the same method as the working example 4. Biotin sign lentile lectin was made to react at a room temperature after 3 times washing with a physiological saline for 1 hour, and the physiological saline washed 5 times. Peroxidase-labeling avidin was made to react for 20 minutes at a room temperature, and spectrometry was carried out by the same method as the working example 3 after 5 times washing with the physiological saline. As a result, the spectrometry value increased linearly like the working example 4 as the non-enzyme treatment sample rate of the test portion increased. This is the result of lentile lectin's recognizing a fucose containing sugar chain, and joining together.
A spectrometry value changed according to a fucose containing sugar chain rate.

[0080]
[Effect of the Invention]
(1) This invention does not need to measure the sugar chain total amount of specific glycoprotein separately, and can provide the measuring method of the rate of the amount of specific sugar chains which can measure simple and promptly the rate of the amount of specific sugar chains over the total amount of sugar chains of specific glycoprotein in a sample.

[0081]The method which will not be able to measure simple and promptly the rate of the amount of specific sugar chains over the total amount of sugar chains of specific glycoprotein without this invention directly is provided.
(2) When the active fragment of the antibody fixed to the solid phase and/or this antibody considers it as the desirable mode of this invention which is an active fragment of an antibody and/or this antibody which does not contain the specific sugar chain used as a measuring object, The measuring method of the specific sugar chain rate over the total amount of sugar chains of wide specific glycoprotein of measurement can be provided that there are few noises.
[0082](3) Urine, blood in which the sample containing specific glycoprotein was extracted, By considering it as the desirable mode of this invention which is one sort chosen from plasma and a blood serum, and is a sample which contains this specific glycoprotein above the concentration which specific glycoprotein combines with all the active fragments of the antibody fixed in the solid phase, and/or this antibody, The measuring method of the specific sugar chain rate over the total amount of sugar chains of specific glycoprotein easily applicable to sick diagnosis can be provided.
[0083](4) When the labeled lectin considers it as the desirable mode of this invention which is the lectin by which the sign was carried out with either an enzyme or biotin, the measuring method of the rate of the amount of specific sugar chains over the total amount of sugar chains of easy specific glycoprotein of detection can be provided by high sensitivity.

[Translation done.]

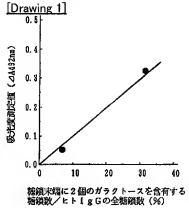
DESCRIPTION OF DRAWINGS

[Brief Description of the Drawings]
[Drawing 1]The figure showing the relation between the absorbance in one working example of this invention, and the specific sugar chain of specific protein.
[Drawing 2]The figure showing the result of having measured healthy person blood serum 4 sample and RA patient's-serum 30 sample by this invention by making a vertical axis into an absorbance.
[Drawing 3]The simple model figure showing a part of reaction process for the measuring method of this invention in model.
[Drawing 4]The simple model figure showing a part of reaction process for explanation of a method given in JP,H5-87814,A in model.
[Explanations of letters or numerals]
1 Solid phase
2 Antibody
3 Glycoprotein
4 The specific sugar chain which can be combined with specific lectin
5 The sugar chain which is not combined with specific lectin
6 Lectin
7 Marker substance

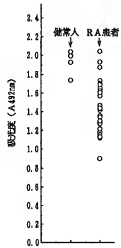
[Translation done.]

DRAWINGS

[Drawing 1]

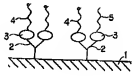


[Drawing 2]



[Drawing 3]

(A)



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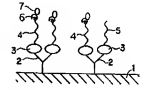
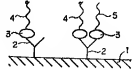


[Drawing 4]

(A)



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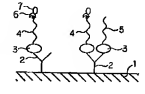

[Translation done.]